Scientific Research Publishing

Advances in Entomology, 2023, 11, 172-187
https://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Lack of Evidence for Local Adaptation of the Endangered Karner Blue Butterfly to Its Sole Larval Hostplant—The Wild Lupine

Kevin Handel, Maria Gabriela Bidart*

Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, USA
Email: *gbidart@bgsu.edu

How to cite this paper: Handel, K. and Gabriela Bidart, M. (2023) Lack of Evidence for Local Adaptation of the Endangered Karner Blue Butterfly to Its Sole Larval Hostplant—The Wild Lupine. *Advances in Entomology*, **11**, 172-187.
https://doi.org/10.4236/ae.2023.113013

Received: June 19, 2023
Accepted: July 24, 2023
Published: July 27, 2023

Abstract

Local adaptation is an important process that drives the evolution of populations within species, and it can be generally expressed by the 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 evaluated whether the federally endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae) is locally adapted to its exclusive larval host plant, the wild lupine (*Lupinus perennis*). To test for local adaptation, individuals from a laboratory-raised colony were reared on wild lupine plants from populations belonging to either their native (Indiana) or a foreign (Michigan and Wisconsin) region. For this purpose, lupine plants from the different populations were grown in a common garden in growth chambers, and one Karner blue larva was placed on each plant. Fitness traits related to growth and development were recorded for each butterfly across populations. Days from hatching to pupation and eclosion showed gender-specific significant differences across wild lupine populations and plant genotypes (within populations). The percent survival of butterflies (from hatching to eclosion) also differed among plants from different populations. These results indicate that wild lupine sources can affect some developmental traits of Karner blue butterflies. However, growth-related traits, such as pupal and adult weight of individuals reared in plants from native populations did not differ from those of foreign regions. 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, future studies including more populations across the geographical range of this butterfly are recommended to evaluate other environmental factors that could influence adaptation on a wider spatial scale.

Keywords

Local Adaptation, Karner Blue Butterfly, Wild Lupine, Butterfly Fitness-Related Responses, Plant Genotypic Effects

1. Introduction

Plant-insect coevolutionary associations are known to drive local adaptation of populations across their geographic range [1] [2]. Spatial heterogeneity of habitat characteristics and biotic factors can cause selection to favor different traits in different populations [3] [4]. Local adaptation is a key mechanism in evolutionary ecology, which results in individuals experiencing increased fitness within their native population compared to foreign populations [5]. 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 [6]. Vayssade *et al.* [7] noted that both "inbreeding and inbreeding depression are key processes in small or isolated populations with implications for the management of threatened or re-introduced organisms". Likewise, Saccheri *et al.* [8] 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 [6]. The existence of local adaptation has been examined across several organisms including (but not limited to) the Chinook salmon *Oncorhynchus tshawtyscha*, the fire salamander *Salamandra salamandra*, the brown trout *Salmo trutta*, the purple sea urchin *Strongylocentrotus purpuratus*, the common frog *Rana temporaria*, and the field cricket *Gryllus campestris* [4] [5] [9] [10] [11] [12]. 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 [5]. In another case related to the host-parasite interaction between the paper wasp *Polistes biglumis* and its parasite *Polistes atrimandibularis,* physical barriers (mountains) did not impede gene flow despite different levels of selective forces operating at low and high altitudes resulting in a "geographic mosaic of co-evolution" [13]. Despite the substantial number of studies on local adaptation, information related to local adaptation in invertebrate specialist herbivores is still scarce; 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" [2].

The interaction between the Karner blue butterfly (KBB) and the wild lupine is an example of a tight coevolutionary association, since this plant is the sole host of the butterfly larvae. The original distribution of the KBB was across 12 states from Minnesota to Maine (USA), and into Ontario (Canada), but it was extirpated from 8 states due to habitat loss associated with fire suppression, agriculture, and urbanization [14]. The KBB became a federally listed endangered species in 1992, after experiencing a total population decline of 99%. This butterfly remained present only in isolated populations in Minnesota, Wisconsin, Michigan and New York, and it was further reintroduced in Indiana, Ohio, and New Hampshire. Current populations remain geographically isolated from one another, which may promote adaptation to particular microclimates [14]. The recovery team also 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 [15] found that a similarly endangered butterfly species endemic to the Willamette Valley of Oregon, *Icaricia icarioides fenderi* (Fender's blue butterfly) had very limited dispersal abilities (0.75 - 2 km) during its total lifetime (approximately 9.5 days). The short lifespan and limited dispersal of these species create a scenario that could lead to local adaptation. Habitat isolation and the dependence of KBB on *Lupinus perennis* (wild lupine) as their only suitable larval food source further enhance the potential for locally adapted populations.

There have been considerable 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, as outlined by the KBB recovery plan team [14]. Reintroduction programs have been carried out at the Oak Openings Region of northwest Ohio and southeast Michigan, the Concord Pine Barrens in Concord (New Hampshire), the Indiana Dunes National Lakeshore and the Albany Pine Bush [16]. The main goal of these programs was to reclassify the species' conservation status by establishing viable metapopulations across the butterfly's original range [14]. Federal reclassification of the species (delisting) would be accomplished when 29 metapopulations (comprised of 13 viable populations ≥ 3000 and 16 large viable populations ≥ 6000) are established within 13 designated recovery units across the butterfly's historic range [14]. However, oftentimes, 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 their interactions [17]. However, there is evidence that KBB populations have been recovering in the Albany Pine Bush Preserve, showing an increase in brood sizes from under 1000 individuals to over 10,000 (depending on the brood) during the period 2007-2018 [18].

The success of a metapopulation system depends on a balance between population dynamics and habitat quality [19]. Dispersal within a metapopulation is a key concern in wildlife conservation and management [15] [20]. Conservation strategies will depend on whether dispersal is positively or negatively associated with the areas of the habitat and patches to be preserved. For example, efforts should be placed to preserve habitats with large patches if they directly influence dispersal; conversely, the highest number of different size patches should be preserved if there is an inverse association with dispersal [20]. Geographically close patches (<2 km) of 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 [14] [15]. In addition to 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 and Pavlovic [21] 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. In a further study, Chau *et al.* [22] found that "the spatial segregation of nectar and host plant resources relative to each other can influence the location and abundance of Karner blues on the landscape".

Although current reintroduction programs may compare habitat characteristics between translocation sites, it is also important to evaluate 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 its sole larval host may amplify its adaptation beyond what a simple habitat analysis could quantify. Maintenance of the habitat across the geographic range of this butterfly and other endangered species is essential for reintroduction programs. The loss of suitable habitats and wildlife corridors due to anthropogenic effects is the main factor causing the loss of populations (and genetic variability) of native species across the geographic ranges of their distribution. Restoring habitat across a species range is the first step towards the recovery of populations to be sustainable in the future [15] [22].

In this study, we assessed whether Karner blue butterflies are locally adapted to distinct populations of wild lupine. This hypothesis was tested by evaluating if this butterfly species would experience decreased fitness when reared on wild lupine plants from foreign populations compared to those of their native habitats. 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 there is evidence of local adaptation, it would be advisable to first evaluate potential matches or mismatches between the source population of butterflies and the target populations of their host plants present in the reintroduction region.

2. Materials and Methods

2.1. Study Organisms

The Karner blue butterfly (*Lycaeides 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 [14]. 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 [19]. 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 are laid by the first-generation hatch during the summer, eggs are laid by the second generation overwinter and hatch during the following spring [14]. Adult Karner butterflies 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 [23]. 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 [24] [25]. The interdependent relationship between the wild lupine and the Karner blue butterfly allows the use of this butterfly as an indicator species to gauge the health of the unique habitats where they coexist. 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 [26].

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,

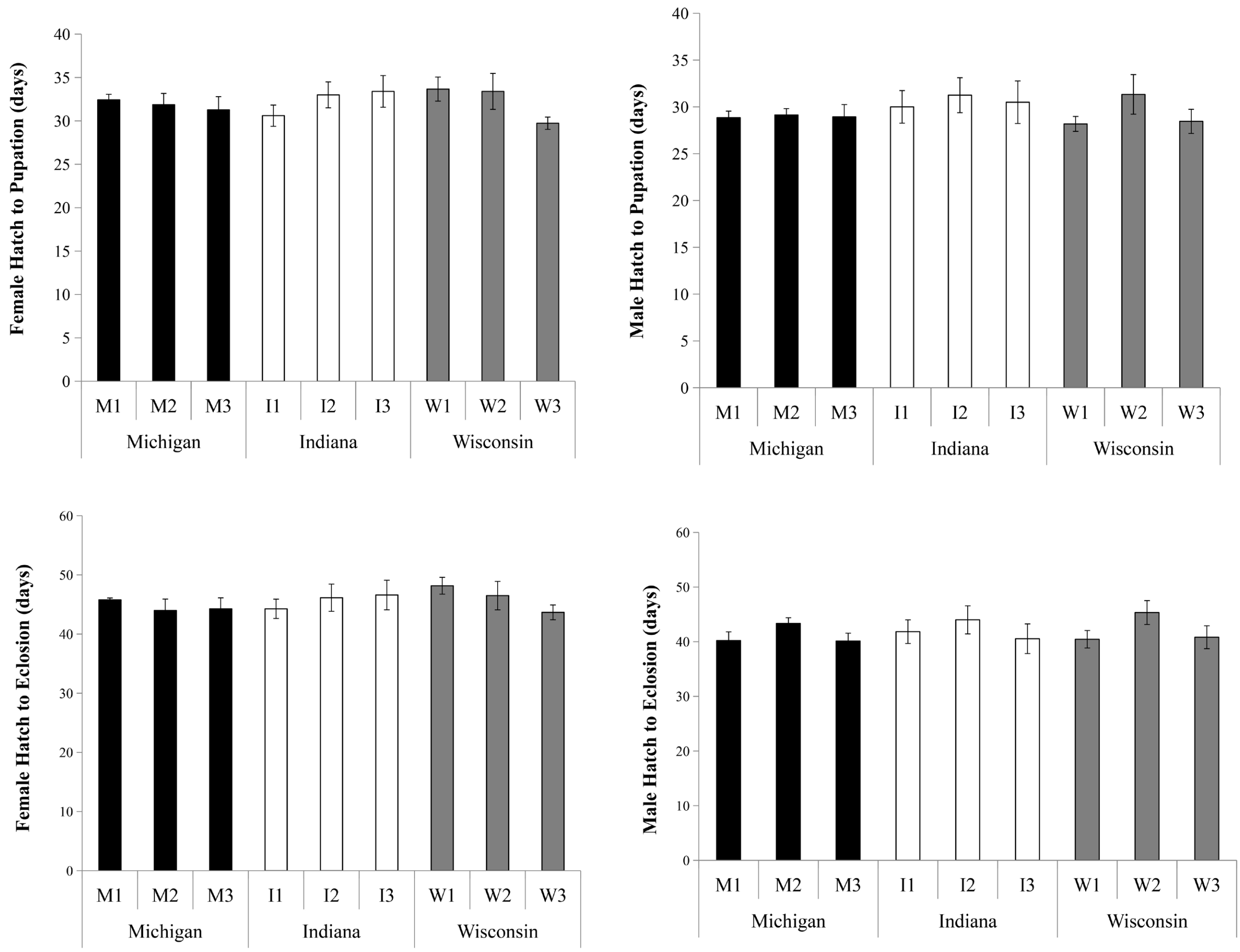

Figure 1. Average number of days (± SE) from egg hatching to pupation and to eclosion for female and male Karner blue butterflies raised on nine different populations of wild lupine.

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.

Karner Blue Butterfly Habitat	Region (state)	County	Distance to Native Habitat (km)	Population
Native	Indiana	LaPorte	≈000	I1 I2 I3
Foreign	Michigan	Allegan	≈140	M1 M2 M3
	Wisconsin	Eau Claire	≈490	W1 W2 W3

lupine seeds were grown 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.

2.2. Experimental Design and Statistical Analyses

To test for local adaptation of the Karner blue butterfly to the wild lupine, a common garden experiment was performed using growth chambers located at Bowling Green State University. A sample of lupine seeds was 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 they were 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. 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. 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) for easier identification of the 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 butterflies perching and fed a 10% honey solution along with regular misting of distilled water.

Several dependent variables were measured for each butterfly throughout its life cycle: larval weight (g), pupal weight (g), adult weight (g), developmental time (*i.e.* days from hatching to pupation and to adult eclosion, and days from pupation to eclosion), and percent survival (from egg hatching to adult eclosion). Gender of each butterfly was recorded. After completion of the experiment, each individual adult was transported back to the University of Notre Dame's captive colony.

The data generated in this experiment was statistically analyzed using SAS (Version 9.1). Analyses of Variance were conducted to evaluate the effects of region, population, plant genotype and gender (and their interactions) on each of the dependent variables (Table 2). Data was transformed when necessary, using inverse and rank transformations. Additionally, survival across population was evaluated using a chi-squared test.

3. Results

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.*

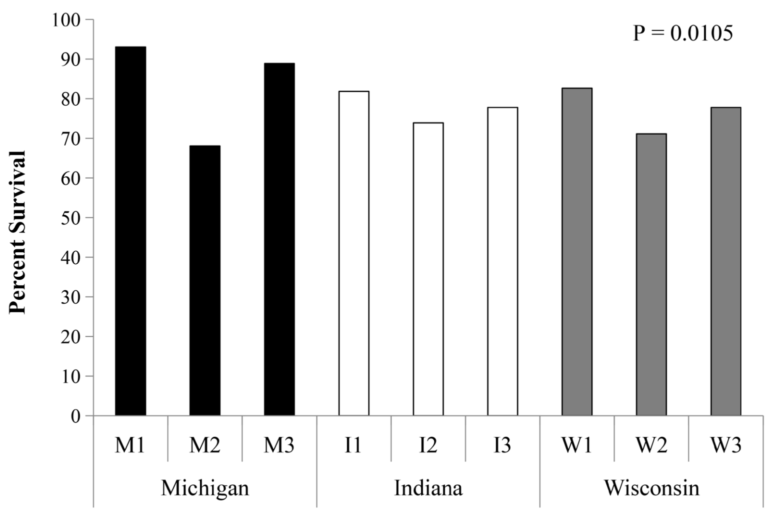

Figure 2. Average percent survival of Karner blue butterflies raised on nine different populations of wild lupine.

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.

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

*P < 0.05; **P < 0.01; ***P < 0.001.

Michigan and Wisconsin) versus when reared on their native Indiana populations. Results of analyses of variance (ANOVA) showed significant differences related to gender and plant genotype and their interaction, but no significant effects of region or population (within region) on the fitness-related variables measured (**Table 2**). There was a statistically significant interaction between genotype (nested in population) by gender for days from egg hatching to both pupation and eclosion (**Table 2**; **Figure 1**). In addition, there were significant differences in survival of individuals raised in the different populations, as shown by a significant Chi-squared test ($P = 0.011$; **Figure 2**). Highest survival was observed for two Michigan lupine populations (M1 and M3).

4. Discussion

Variation in Karner blue butterflies' measured traits was based on gender and plant genotype (within populations). 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 plant genotype suggest the evolution of adaptation in the Karner blue butterfly may be more influenced by wild lupine intra-population genetic differences than by plants' geographic origin. The significant plant genotype by gender interaction for developmental variables (days from hatching to pupation and eclosion) is important because it may influence survival and mating success. It was interesting to find that survival of the butterflies was maximized in two "foreign" Michigan populations (M1 and M3). This result is partially contrary to the expression of local adaptation because it would be expected the butterflies would have higher survival when raised in plants from their place of origin (Indiana).

5. Conclusions

Overall, experimental results did not 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, and New York—while populations in two states—Ohio and New Hampshire—are comprised solely of non-native reintroduced individuals [14]. 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. Therefore, it is possible that local adaptation is evident only on a broader scale than our selected 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 could be related to an environmental source of variation independent of geographic region. If the butterfly source population is adapted to a particular wild lupine chemical (alkaloid) profile determined by 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 would 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 [27] 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 (≥300 m from host tree); however, there was no significant difference when raised on neighboring trees (<5 m to host tree) [27]. In a study by Bischoff *et al.* [28], 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 as 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 [7] [8] [29] [30]. Franke and Fischer [29] showed that the tropical butterfly *Bicyclus anynana* experienced reduced fitness even with relatively low levels of inbreeding. Additionally, some 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 may suggest the effects of inbreeding. 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 [31]. Therefore, the Karner blue butterfly may be particularly susceptible to captive inbreeding stemming from the loss of 99% of wild populations combined with a two-generation per year life cycle. According to Rollinson *et al.* [31], this scenario dictates a management choice between perpetuating genetic homogeneity, or introducing new, genetically diverse individuals, but 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. The possibility that consistent collections from native populations may create negative consequences in an already fragmented and delicate endangered wild population may counteract the intention of reintroduction programs [7] [8] [32] [33] [34] [35]. Lastly, potential maternal effects could have also been an additional factor influencing the results of this study. It has been widely acknowledged that maternal effects can greatly influence phenotypic variation [36] [37] [38] [39]. If previous generations were not consistently reared on wild lupine from Indiana Dunes National Lakeshore; then, it is possible that the individuals used in this study would not express significant adaptation to the plant populations classified here as native.

The 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 [34]. 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 25 - 30 years [40]. 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 local 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 [34]. 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 [41] estimated local adaptation occurred in the invasive California poppy (*Eschscholzia californica*) 110 - 150 years after its introduction to Chile.

The 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 butterflies could maintain consistent fitness levels when they were geographically translocated to restoration sites. However, this scenario may change when assessing populations other than the studied ones or beyond selected regions. 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 lupine habitat surrounding 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, the 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.

The potential for a 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 [16]. Since species relisting standards were set in 2003, only three Karner blue butterfly populations have met the criteria to be considered a viable metapopulation [16]. However, more recent evidence of KBB population recovery has been documented in the Albany Pine Bush Preserve during the period between 2007 and 2018 [18].

As habitat loss inevitably increases and intensifies habitat fragmentation, populations of endangered species can become increasingly more isolated and reduce population resilience. Additionally, impending climate change is predicted to have harmful consequences on remaining KBB populations [42] [43] [44]. 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 prior local adaptation. Even though no clear evidence of local adaptation was found in this study, there were some differences in the performance of the butterfly in different populations, in terms of both survival and developmental time. These differences may still be important to consider in the potential conservation-related management of this species. Future evaluations of the potential match between donor butterfly populations and receiving wild lupine habitats would be essential for the success of re-introduction programs and the recovery of this species.

Acknowledgements

We are grateful to Jessica J. Hellmann (University of Notre Dame) for providing larvae of the Karner blue butterfly to conduct this study in Dr. Bidart's lab at BGSU (U.S. Fish & Wildlife Service Permit TE43555A-1). This work was funded by an NSF grant (IOS-0715220) awarded to MGB.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ehrlich, P.R. and Raven, P.H. (1964) Butterflies and Plants: A Study in Coevolution. *Evolution*, **18**, 586-608. https://doi.org/10.2307/2406212

[2] Cogni, R., Trigo, J.R. and Futuyma, D.J. (2011) Varying Herbivore Population Structure Correlates with Lack of Local Adaptation in a Geographic Variable Plant- Herbivore Interaction. *PLOS ONE*, **6**, e29220.

https://doi.org/10.1371/journal.pone.0029220

[3] Kalske, A., Muola, A., Laukkanen, L., Mutikainen, P. and Leimu, R. (2012) Variation and Constraints of Local Adaptation of a Long-Lived Plant, Its Pollinator and Specialist Herbivores. *Journal of Ecology*, **100**, 1359-1372. https://doi.org/10.1111/j.1365-2745.2012.02008.x

[4] Tregenza, T., Rodríguez-Muñoz, R., Boonekamp, J.J., *et al*. (2021) Evidence for Genetic Isolation and Local Adaptation in the Field Cricket *Gryllus campestris*. *Journal of Evolutionary Biology*, **34**, 1624-1636. https://doi.org/10.1111/jeb.13911

[5] Fraser, D.J., Weir, L.K., Bernatchez, L., Hansen, M.M. and Taylor, E.B. (2011) Extent and Scale of Local Adaptation in Salmonid Fishes: Review and Meta-Analysis. *Heredity*, **106**, 404-420. https://doi.org/10.1038/hdy.2010.167

[6] Jourdan-Pineau, H., Folly, J., Crochet, P. and David, P. (2012) Testing the Influence of Family Structure and Outbreeding Depression on Heterozygosity-Fitness Correlations in Small Populations. *Evolution*, **66**, 3624-3631. https://doi.org/10.1111/j.1558-5646.2012.01705.x

[7] Vayssade, C., Fazio, C., Quaglietti, B., Auguste, A., Ris, N. and Fauvergue, X. (2014) Inbreeding Depression in a Parasitoid Wasp with Single-Locus Complementary Sex Determination. *PLOS ONE*, **9**, e97733. https://doi.org/10.1371/journal.pone.0097733

[8] Saccheri, L., Kuussaari, M., Kankare, M., Vikmann, P., Fortelius, W. and Hanski, L. (1998) Inbreeding and Extinction in a Butterfly Metapopulation. *Nature*, **392**, 491-494. https://doi.org/10.1038/33136

[9] Maneti, R. and Ficetola, G.F. (2013) Salamanders Breeding in Subterranean Habitats: Local Adaptations or Behavioral Plasticity? *Journal of Zoology*, **289**, 182-188. https://doi.org/10.1111/j.1469-7998.2012.00976.x

[10] Pespeni, M.H., Chan, F., Menge, B.A. and Palumbi, S.R. (2013) Signs of Adaptation to Local pH Conditions across an Environmental Mosaic in the California Current Ecosystem. *Integrative and Comparative Biology*, **53**, 857-870. https://doi.org/10.1093/icb/ict094

[11] Meier, K., Hansen, M.M., Normandeau, E., Mensberg, K.D., Frydenberg, J., Larsen, P., Foged, B. and Dorte, B. (2014) Local Adaptation at the Transcriptome Level in Brown Trout: Evidence from Early Life History Temperature Genomic Reaction Norms. *PLOS ONE*, **9**, e85171. https://doi.org/10.1371/journal.pone.0085171

[12] Muir, A.P., Biek, R., Thomas, R. and Mable, B.K. (2014) Local Adaptation with High Gene Flow: Temperature Parameters Drive Adaptation to Altitude in the Common Frog (*Rana temporaria*). *Molecular Ecology*, **23**, 561-574. https://doi.org/10.1111/mec.12624

[13] Seppä, P., Bonelli, M., Dupont, S., Hakala, S.M., Bagnères, A.G. and Lorenzi, M.C. (2020) Strong Gene Flow Undermines Local Adaptations in a Host Parasite System. *Insects*, **11**, Article 585. https://doi.org/10.3390/insects11090585

[14] Karner Blue Butterfly Recovery Team (2003) Karner Blue Butterfly (*Lycaeides melissa samuelis*) Recovery Plan. The Department of Interior U.S. Fish and Wildlife Service.

[15] Schultz, C.B. (1998) Dispersal Behavior and Its Implications for Reserve Design in a Rare Oregon Butterfly. *Conservation Biology*, **12**, 284-292.

[16] U.S. Fish and Wildlife Service, Department of the Interior (2012) Karner Blue Butterfly (*Lycaeides melissa samuelis*) 5-Year Review: Summary and Evaluation. Ecological Services Field Office, New Franken.

[17] Brown, R.D., Kenny, N. and Corry, R. C. (2011) Testing the Microclimatic Habitat

Design Framework in Abandoned Sand and Gravel Extraction Sites Using the Karner Blue Butterfly. *Ecological Restoration*, **29**, 52-63. https://doi.org/10.3368/er.29.1-2.52

[18] Campbell, S.P., Dillon, A.M. and Gifford, N.A. (2018) Population Trends of the Karner Blue (*Plebejus melissa samuelis*) in the Albany Pine Bush Recovery Unit, 2007-2018: A Short Report to the Albany Pine Bush Preserve Commission, New York State Department of Environmental Conservation, and New York Field Office of the United States Fish and Wildlife Service. https://www.townofguilderland.org/sites/g/files/vyhlif7546/f/uploads/apbp_kbb_monitoring_short_report_2007-2018-minus_maps.pdf

[19] Lane, C.P. and Andow, D.A. (2003) Oak Savanna Subhabitat Variation and the Population Biology of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae). *Entomological Society of America*, **96**, 799-809. https://doi.org/10.1603/0013-8746(2003)096[0799:OSSVAT]2.0.CO;2

[20] Wang, S. and Altermatt, F. (2019) Metapopulations Revisited: The Area-Dependence of Dispersal Matters. *Ecology*, **100**, e02792. https://doi.org/10.1002/ecy.2792

[21] Grundel, R. and Pavlovic, N.B. (2007) Resource Availability, Matrix Quality, Microclimate, and Spatial Pattern as Predictors of Patch Use by the Karner Blue Butterfly. *Biological Resoration*, **135**, 135-144. https://doi.org/10.1016/j.biocon.2006.10.003

[22] Chau, S.N., Bristow, L.V., Grundel, R. and Hellmann, J.J. (2020) Resource Segregation at Fine Spatial Scales Explains Karner Blue Butterfly (*Lycaeides melissa samuelis*) Distribution. *Journal of Insect Conservation*, **24**, 739-749. https://doi.org/10.1007/s10841-020-00244-0

[23] Gibbs, J.P., Smart, L.B., Newhouse, A.E. and Leopold, D.J. (2012) A Molecular and Fitness Evaluation of Commercially Available versus Locally Collected Blue Lupine *Lupinus perennis* L. Seeds for Use in Ecosystem Restoration Efforts. *Restoration Ecology*, **20**, 456-461. https://doi.org/10.1111/j.1526-100X.2011.00809.x

[24] King, R.S. (2003) Habitat Management for the Karner Blue Butterfly (*Lycaeides melissa samuelis*): Evaluating the Short-Term Consequences. *Ecological Restoration*, **21**, 101-106. https://doi.org/10.3368/er.21.2.101

[25] Forrester, J.A., Leopold, D.J. and Hafner, S.D. (2005) Maintaining Critical Habitat in a Heavily Managed Landscape: Effects of Power Line Corridor Management on Karner Blue Butterfly (*Lycaeides melissa samuelis*) Habitat. *Restoration Ecology*, **13**, 488-498. https://doi.org/10.1111/j.1526-100X.2005.00061.x

[26] Wood, E.M., Pidgeon, A.M., Gratton, C. and Wilder, T.T. (2011) Effects of Oak Barrens Habitat Management for Karner Blue Butterfly (*Lycaeides samuelis*) on the Avian Community. *Biological Conservation*, **144**, 3117-3126. https://doi.org/10.1016/j.biocon.2011.10.010

[27] Hanks, L.M. and Denno, R.F. (1994) Local Adaptation in the Armored Scale Insect *Pseudaulacaspis pentagona* (Homoptera: Diaspididae). *Ecology*, **75**, 2301-2310. https://doi.org/10.2307/1940885

[28] Bischoff, A., Cremieux, L., Smilauerova, M., Lawson, C.S., Mortimer, S.R., Dolezal, J., Lanta, V., Edwards, A.R., Brook, A.J., Macel, M., Leps, J., Steinger, T. and Muller-Scharer, H. (2006) Detecting Local Adaptation in Widespread Grassland Species—The Importance of Scale and Local Plant Community. *Journal of Ecology*, **94**, 1130-1142. https://doi.org/10.1111/j.1365-2745.2006.01174.x

[29] Franke, K. and Fischer, K. (2013) Effects of Inbreeding and Temperature Stress on Life History and Immune Function in a Butterfly. *Journal of Evolutionary Biology*,

26, 517-528. https://doi.org/10.1111/jeb.12064

[30] Liu, X., Tu, X., He, H., Chen, C. and Xue, F. (2014) Evidence for Inbreeding Depression and Pre-Copulatory, But Not Post Copulatory Inbreeding Avoidance in the Cabbage Beetle Colaphellus bowringi. *PLOS ONE*, **9**, e94389. https://doi.org/10.1371/journal.pone.0094389

[31] Rollinson, N., Keith, D.M., Houde, A.S., Debes, P.V., McBride, M.C. and Hutchings, J.A. (2014) Risk Assessment of Inbreeding and Outbreeding Depression in a Captive-Breeding Program. *Conservation Biology*, **28**, 529-540. https://doi.org/10.1111/cobi.12188

[32] Sletvold, N., Grindeland, J.M., Zu, P. and Agren, J. (2012) Strong Inbreeding Depression and Local Outbreeding Depression in the Rewarding Orchid *Gymnadenia conopsea*. *Conservation Genetics*, **13**, 1305-1315. https://doi.org/10.1007/s10592-012-0373-7

[33] Long, T.A.F., Rowe, L. and Agrawal, A.F. (2013) The Effects of Selective History and Environmental Heterogeneity on Inbreeding Depression in Experimental Populations of *Drosophila melanogaster*. *The American Naturalist*, **181**, 532-544. https://doi.org/10.1086/669675

[34] Angeloni, F., Vergeer, P., Wagemaker, C.A.M. and Ouborg, N.J. (2014) Within and between Population Variation in Inbreeding Depression in the Locally Threatened Perennial *Scabiosa columbaria*. *Conservation Genetics*, **15**, 331-342. https://doi.org/10.1007/s10592-013-0541-4

[35] Brzeski, K.E., Rabon, D.R., Chamberlain, M.J., Waits, L.P. and Taylor, S.S. (2014) Inbreeding and Inbreeding Depression in Endangered Red Wolves (*Canis rufus*). *Molecular Ecology*, **23**, 4241-4255. https://doi.org/10.1111/mec.12871

[36] Roach, D.A. and Wulff, R.D. (1987) Maternal Effects in Plants. *Annual Review of Ecology and Systematics*, **18**, 209-235. https://doi.org/10.1146/annurev.es.18.110187.001233

[37] Mousseau, T.A. and Dingle, H. (1991) Maternal Effects in Insect Life Histories. *Annual Review of Ecology and Systematics*, **36**, 511-534. https://doi.org/10.1146/annurev.en.36.010191.002455

[38] Bernardo, J. (1996) Maternal Effects in Animal Ecology. *American Zoology*, **36**, 83-105. https://doi.org/10.1093/icb/36.2.83

[39] Mousseau, T.A. and Fox, C.W. (1998) The Adaptive Significance of Maternal Effects. *Trends in Ecology & Evolution*, **13**, 403-407. https://doi.org/10.1016/S0169-5347(98)01472-4

[40] U.S. Fish and Wildlife Service, Department of the Interior (1992) Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Karner Blue Butterfly. *Federal Register*, **57**, 59236-59244.

[41] Leger, E.A. and Rice, K.J. (2007) Assessing the Speed and Predictability of Local Adaptation in Invasive California Poppy (*Eschscholzia californica*). *Journal of Evolutionary Ecology*, **20**, 1090-1103. https://doi.org/10.1111/j.1420-9101.2006.01292.x

[42] Swengel, S.R., Schlicht, D., Olsen, F. and Swengel, A.B. (2011) Declines of Prairie Butterflies in the Midwestern USA. *Journal of Insect Conservation*, **15**, 327-339. https://doi.org/10.1007/s10841-010-9323-1

[43] Hoving, C.L., Lee, Y., Badra, P.J. and Klatt, B.J. (2013) Changing Climate, Changing Wildlife a Vulnerability Assessment of 400 Species of Greatest Conservation Need and Game Species in Michigan. Michigan Department of Natural Resources, Wildlife Division Report No. 3564.

[44] National Park Service, U.S. Department of the Interior (2014) Impact of Climate Change on the Karner Blue Butterfly. Indiana Dunes National Lakeshore, National Park Service.