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ISLAND POPULATIONS AND TRAIT COMPARISONS OF TIGER SWALLOWTAIL BUTTERFLIES, P. CANADENSIS, IN THE GREAT LAKES REGION

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Gabriel J. Ording

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Lawrence Bessar
J. Mark Scriber
Major professor

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By

Gabriel J. Ording

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ABSTRACT

ISLAND POPULATIONS AND TRAIT COMPARISONS OF TIGER SWALLOWTAIL BUTTERFLIES, *P. CANADENSIS*, IN THE GREAT LAKES REGION

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The objectives of this thesis were to examine gene flow between geographically isolated Great Lakes Island subpopulations of swallowtail butterflies, *Papilio canadensis*, using wing trait morphometrics and allozyme electrophoresis. Specimens from Isle Royale in Lake Superior, Beaver and South Manitou Islands in Lake Michigan, were compared to adjacent mainland populations. There were no significant differences between either Isle Royale or Beaver Island and their respective adjacent mainland populations. South Manitou Island however, showed significant differences from adjacent mainland populations for every character analyzed. These differences were attributed to an introgression of genes from *Papilio glaucus*, a closely related species who's described range begins approximately 150 km to the south.

The extent of the *P. glaucus* introgression on and around South Manitou Island was investigated through further analysis of morphometric, biochemical, behavioral, and physiological traits. The Tiger Swallowtail butterflies on and around South Manitou exhibit many characteristics making them appear hybrid-like, intermediate between *P. canadensis* and *P. glaucus*. It is suggested that periods of increased thermal unit accumulations along the western shore of Michigan may allow extended movement of *P. glaucus* alleles significantly further northward from that observed inland.

To my family

ACKNOWLEDGMENTS

Thanks to my co-major advisors, Dr. Mark Scriber for his never ending supply of enthusiasm, guidance, patience and understanding; Dr. Larry Besaw for many years of mentorship in the realms of science education. Thanks to my guidance committee, Dr. Cathy Bristow, and Dr. Jim Miller for their willingness to participate in this project and for providing helpful suggestions and revisions. Thanks also to Drs. Jim Smith and Guy Bush for welcoming me into their laboratory to conduct the biochemical aspects of this study.

Thanks to the National Park Service, at both Isle Royale and Sleeping Bear Dunes, for allowing this research to take place, providing special use and specimen collection permits. Special thanks to Jack Oelfke and Steve Yancho, the resource management staff at both parks respectively.

Thanks to all of the graduate students and lab technicians of the Scriber Lab over the past several years, for everything from showing me the ropes in the very beginning to helping me with the finite details near the end. Thank you for the moral support and the friendship. Special thanks to Aram Stump for an incredible amount of help with various aspects of this research, especially sharing his expertise in allozyme electrophoresis.

Great gobs of thanks to my wife Betsy, for her patience and love.

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CHAPTER 1:

INTRODUCTION

Gene flow is the movement of gametes, individuals, or groups of individuals from one place to another (Slatkin 1987). Two important ecological concepts that are significantly impacted by levels of organismal gene flow are island biogeography and hybrid zone theory. This thesis investigates aspects of both of these theories, looking at populations of Papilionidae butterflies in the Great Lakes region.

Islands are important in helping biologists understand the processes of evolution and natural selection. Visiting the Galapagos Islands was of paramount importance in Charles Darwin's development of his theories. Likewise, it was while visiting the island of Ternate (Indonesia), that Alfred Wallace was struck by the idea of evolution (Williamson 1981). Since then, island systems have been investigated extensively, and used as natural laboratories, to further understand these processes. Key research conducted by MacArthur and Wilson (1967) investigated aspects of island biogeography correlating island sizes and distances from the adjacent mainland, with rates of species colonization and extinction. Of course these rates vary for different organisms due to differing dispersal mechanisms.

Island populations of organisms live in geographic isolation from their mainland counterparts. This isolation can act as a barrier to gene flow between the island and the mainland populations. The island population has a limited number of individuals contributing to its gene pool, and is subject to genetic drift. Genetic drift is the unpredictable and random change in gene frequency due to finite population size (Slatkin

1987). Genetic drift can act as a mechanism to drive genetic differentiation between island and mainland populations.

As with island biogeography theory, the concept of gene flow heavily influences hybrid zone theory. A hybrid zone is a geographic region where the populations of two different species overlap and cross producing offspring of mixed ancestry (Harrison 1993). The width of a hybrid zone is determined by the counterbalancing forces of selection and dispersal. There is great debate as to the importance that should be ascribed to hybridization in evolutionary processes. Frequently, hybrid offspring are far less fit than either parental strain, sometimes even sterile. However, hybridization may also provide unique combinations of alleles that in certain environments prove superior to either parent strain (Arnold & Hodges 1995).

A great deal of research has been conducted on dispersal and the levels of gene flow in and between populations of many species of butterflies. Great variation exists in the tendency of different butterflies to travel. Monarch butterflies (*Danaus plexippus*) are well known for their annual migration from various locations in North America 2000 miles south to Mexico. Assisted by winds, monarchs have been known to fly 80 miles in a single day. But, monarchs are the only species in their family known to migrate (Wolfe 1994). On the other hand, after many years of rigorous research it has been documented that the Checkerspot Butterfly (*Euphydryas editha*) possesses intrinsic barriers to widespread dispersal (Ehrlich 1961). "Butterflies (except those few species which are migratory) seem to be quite sedentary as compared with what one might expect in view of their powers of movement." (Ehrlich and Raven 1969).

Barriers to gene flow in various Lepidoptera have been described, taking several forms. Three closely related species of saturniid silk moths *Callosomia promethea, C. angulifera, and C. securifera* can be hybridized by hand-pairing but are reproductively isolated in nature by temporal differences in mating times (Johnson et al. 1996). More commonly described as limiting gene flow are physical geographic barriers between populations of the same species. For example, some checkerspot butterfly populations in mountainous areas have restricted gene flow (Britten et al. 1995). Also, the Great Lakes themselves have been described as barriers reducing gene flow between populations of butterflies (Waldbauer & Sternburg 1988).

There have been investigations of dispersal and gene flow done on species of *Papilionidae.* Using methods of mark, release, and recapture, on Maryland populations of *Papilio glaucus,* it was concluded that males tend not to disperse but that females do tend to disperse more widely (Fales 1959). More recent research utilizing molecular markers suggests high gene flow between populations of several *Papilio* species: *P. hospiton* (Aubert et al. 1997), *P. machaon* (Auber et al. 1997, Hoole et al. 1999), and *P. glaucus* (Bossart & Scriber 1995).

In the Great Lakes region a great deal of research has been conducted investigating gene flow between populations of *Papilio glaucus* and *P. canadensis*, within their respective ranges and across their narrow hybrid zone (Scriber 1990, Hagen et al. 1991, Scriber 1994, Deering 1998, Scriber et al. 2001). An investigation was conducted in Michigan solely on populations of *P. canadensis* to determine whether there was restricted gene flow across the state. It was concluded that there were high levels of gene flow between the populations sampled (Stump 2000).

This thesis reports an investigation of gene flow between island and mainland populations of *P. canadensis* in the Great Lakes. Chapter 2 emphasizes the initial findings. Early in this investigation, high levels of genetic introgression from *P. glaucus* were found in the South Manitou Island population of *P. canadensis*. This highly unexpected finding shifted the long-term emphasis of this research project. Chapter 3 reports follow up research that investigated the extent to which introgression is present on and around the South Manitou Island population of *P. canadensis*.

CHAPTER 2:

ARE ISLAND POPULATIONS OF *PAPILIO CANADENSIS* ISOLATED FROM ADJACENT MAINLAND POPULATIONS?

Introduction

Island biogeography theory suggests that island populations experiencing reduced levels of immigration have a tendency to become genetically differentiated from their mainland counterparts (Johnson et al. 2000). This genetic differentiation can be the result of a combination of mechanisms, including founder effect, genetic drift due to finite population size, or natural selection favoring adaptations to local environmental conditions. These mechanisms leading to genetic differentiation can be counterbalanced by gene flow (Slatkin 1987).

A great deal of research has been conducted on various species of Papilionidae butterfly populations, including investigations of dispersal, gene flow, and estimations of population differentiation (Fales 1959, Tong & Shapiro 1989, Bossart & Scriber 1995, Aubert et al. 1997, Hoole et al. 1999, Stump 2000). Each of the investigations measuring gene flow between populations suggested that there were sufficient levels of gene flow to counterbalance genetic differentiation. However, none of these investigations sampled island populations of Papilionidae.

Determination of genetic differentiation between island and mainland populations can be accomplished using various techniques for trait analysis. One method to expose divergence is statistical analysis of heritable quantitative phenotypic characteristics (Boag & van Noordwijk 1987). However, a more powerful technique to identify genetic

divergence is the analysis of biochemical markers. Enzyme electrophoresis has become a popularly utilized method to estimate levels of genetic variation and differentiation (McKechnie et al. 1975, Leberg 1992, Bossart & Scriber 1995, Stump 2000). Combining these two techniques can prove a powerful method by which to identify island and mainland population genetic differentiation.

Butterfly forewing length is a direct indicator of adult size. This can be influenced by host plant nutritional quality, impacted by thermal environmental conditions, and has also been shown to be commonly selected upon in island populations of insects. It has been shown that *P. canadensis* living in the interior of Alaska endure significantly shorter, cooler summers than those living in northern Michigan. As adaptations to these differing local environmental conditions, *P. canadensis* living in Alaska were shown to lay smaller clutches of eggs but each egg a larger size, resulting in larger first instar larvae, which then were observed to have 40% higher consumption rates, that led to earlier pupation. These Alaskan pupae were significantly smaller, which in turn produced smaller adult size and forewing length, than adults found in northern Michigan (Ayres & Scriber 1994). It has been documented that the climatic conditions of the three island locations under investigation in this thesis, are heavily moderated by their respective surrounding bodies of water (Hatt et al. 1948, Allen 1979, Haswell & Alanen 1994). This could serve to act differentially for Great Lakes island versus mainland populations, as a selective agent on the forewing length character.

Sometimes a reduction or complete loss of wings is a common insect adaptation to island living. There are many examples of beetle (Coleoptera) populations living in

isolation on islands or mountain tops around the world, that are reported to exhibit atrophy or complete loss of their wings (Darlington 1943). Tristan da Cunha, a group of small volcanic islands in the South Atlantic Ocean, is home to 20 endemic species of beetles, all but two of which have reduced wings, and also a flightless species of Drosophilid (*Scaptomyza frustolifera*) (Williamson 1981).

Banding patterns on butterfly wings is a common method by which species can be distinguished, as is the case between *Papilio glaucus* and *P. canadensis* (Hagen et al. 1991), or can also be used to discern differences between populations within a species. The Monarch butterfly (*Danaus plexippus*) has a range that extends across the Western Hemisphere, from Alaska in the north to Patagonia in the south. There are differences in wing banding patterns that allow individuals coming from one region to be distinguished from an individual coming from another (Williams et al. 1942).

P. canadensis has a distinct black band on its hind wing that partially fills the anal cell (Fig. 2.3). This morphologic character is one used to distinguish between *Papilio canadensis* and *P. glaucus* (Hagen et al. 1991). This dark band is wider in the northern of the two species, and it has been suggested that increased dark melanic coloration could serve as a thermal collection mechanism, helping increase possible metabolic rates, in colder environments (Watt 1968, Kingsolver 1985, 1987, 1995). Again, the island populations under investigation in this thesis exist under differing thermal climactic regimes than do their mainland counter parts, owing to the lake effect. This could act differentially, as a selective force on the black bandwidth, between island and mainland populations.

Analysis of allozyme allele frequencies is extremely useful in identifying the presence of genetic differentiation between populations. This can be especially true when populations under scrutiny are of a finite size located on islands. Rare alleles that might arise due to mutation can be amplified on islands, due to genetic drift. Also, there is a tendency in isolated locations with finite effective population sizes, towards a decrease in average heterozygosity and loss of alleles at any given locus (Carlquist 1974, Jacquard 1974, Hartl 1988, Grant 1998). The most informative allozyme loci to use for such an investigation would be those that are highly polymorphic. *P. canadensis* populations were assessed for differentiation using the Pgd (6-Phosphogluconate dehydrogenase) allozyme locus. This locus was chosen as a result of it being highly polymorphic (Hagen & Scriber 1991), relatively consistent and easy to interpret, and was highly informative for the latter portion of this thesis.

MATERIALS AND METHODS

Papilio canadensis is an extremely common butterfly in the Great Lakes region, including robust populations found on several of the islands of the Great Lakes. In order to determine whether there was significant genetic differentiation between these island populations and their mainland counterparts, samples were collected from three islands (all at least 12 km from the nearest "mainland") and five adjacent mainland locations. Morphometric analyses were performed on two wing characteristics, forewing lengths and hind wing black bandwidths. Multiple statistical analyses were performed to identify significant differences. Allozyme electrophoresis analyses were used to determine allele and genotype frequencies at a highly polymorphic enzyme locus (Pgd; phosphogluconate dehydrogenase).

Specimen Acquisition and Transport

Papilio specimens utilized in this research were live-captured by net, from selected wild populations throughout the State of Michigan (unless otherwise noted). Specimens were most frequently captured while feeding on available sources of nectar, puddling, and sometimes while in flight. Specimen collections were primarily made during mid-day, between approximately ten o'clock a.m. and four o'clock p.m. on warm sunny days, these being the predominant hours for flight activity. Mainland specimen collections were made using vehicle transportation. Butterflies were located along the side of the road, puddling, in flight, or fluttering on nectar sources. This method of specimen collection often led to the discovery of desirable puddling locations or high

concentrations of appropriate nectar sources, each often times with high densities of nectaring butterflies. These locations could then be returned to multiple times in a season for further collection.

Collections on island locations were similarly made, however foot travel was the only available mode of transportation. Butterfly specimens were encountered while hiking across the island locations. Again, specimens were found in flight, puddling, or on nectar sources. Locations that offered high concentrations of butterfly activity due to appropriate puddling conditions or high concentrations of nectar sources were returned to, sometimes multiple times in a day.

The dates and locations of specimen collections for 1998 are as follows (see associated map Figure 2.1): Cook Co. MN (n = 35 males; 29 May and 23 June), Isle Royale National Park, Keweenaw Co. (n = 28 males, n = 1 female; 23-28 May), Gogebic Co. (n = 36 males, n = 4 females; 22 and 28 May), Dickinson Co. (n = 63 males, n = 19 females; 8 June), Beaver Island, Charlevoix Co. (n = 11 males; 14 June), Charlevoix Co. (mainland) (n = 50 males, n = 17 females; 14-25 May), South Manitou Island, Leelenau Co. (n = 32 males, n = 24 females; 17-18 June), Mason Co. (n = 50 males, n = 15 females; 23 May). The difference in the numbers of males collected compared to females collected is likely the result of two factors. First, collections may have been completed early in the flight period of the species. These earlier stages of annual flight are generally dominated by males for which eclosion occurs somewhat earlier. The other likely reason for the bias towards male sampling is the result of collecting large numbers of specimens while they are puddling. Puddling is an activity that is performed almost exclusively by

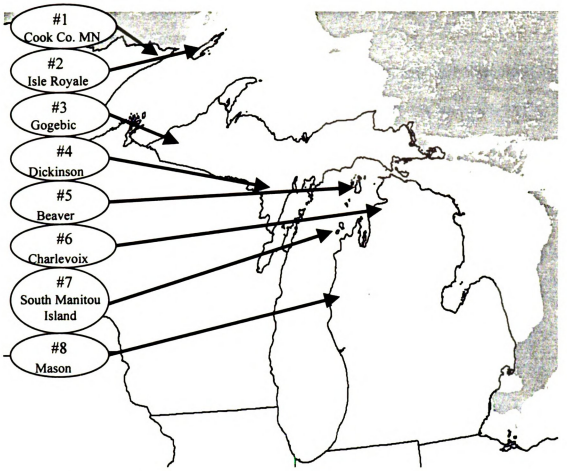


Figure 2.1. 1998 Sample sites and sample sizes.

Sample Location	Males	Females
1. Cook Co., Minnesota	35	0
2. Isle Royale National Park, MI	28	1
3. Gogebic Co., Michigan	36	4
4. Dickinson Co., Michigan	63	19
5. Beaver Island, Michigan	11	3
6. Charlevoix Co., Michigan	50	17
7. South Manitou Island, Michigan	32	24
8. Mason Co., Michigan	50	15

males. It is thought that the purpose for puddling is that males are collecting salts and nutrients required for sperm production.

Upon capture, individual specimens were placed with their wings folded back into 2 oz. Glassine envelopes, which were appropriately labeled with specimen sex, date and location of capture. Collections were transported alive to the laboratory in Tupperware® plastic containers in ice coolers, which lowered specimen body temperatures and slowed metabolism. While at the remote locations of South Manitou Island and Isle Royale National Park, lowering of specimen body temperatures was accomplished by placing the Tupperware® containers into large airtight zip-lock food storage bags. These were then placed into a collapsible bucket containing cool water from either Lake Michigan or Lake Superior. Isle Royale National Park specimens were overnight delivered (live) by the U.S. Postal Service, from Houghton-Hancock to East Lansing, Michigan. Upon arrival in East Lansing, specimens were preserved by freezing them alive in an -80° C ultra-low biological freezer for later processing.

Wing Morphometrics

After the wings were detached from adult swallowtail specimens during the allozyme electrophoresis preparatory protocol, they were assayed for two major morphological features. Forewing length, from the distal tip of the wing to the basal thoracic attachment (Fig. 2.2), was measured using a clear plastic metric ruler to the nearest mm. On the ventral surface of the wings, the width of the anal black band was assessed as a percentage of the distance from the wing edge to the Cu2 vein. This measurement was taken at a line of intersection with the junction of vein Cu2 and the

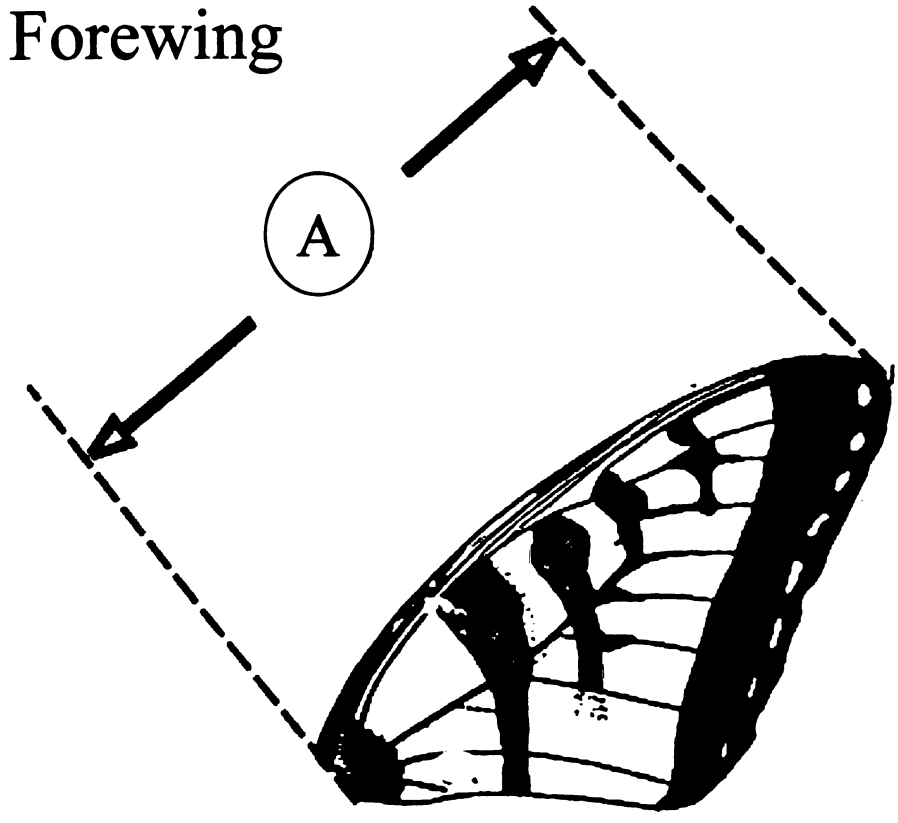


Figure 2.2. Forewing length measurements (measurement A) are the distance from the tip of the forewing to the thoracic wing base attachment (figure modified from Leubke et al. 1988).

discal cell (Fig. 2.3). This anal black band measurement was taken to the nearest .1mm using a dissecting microscope and a WILD glass micrometer slip. For both of these morphometric characters assayed, measurement values for both wings were taken when available, and the mean values for each individual have been utilized for analysis. In cases where wings were damaged and ripped, preventing an accurate measurement; if one wing was undamaged a single measurement has been utilized for analysis; if both wings were damaged, the specimen has not been included in the analyses.

Allozyme Electrophoresis

Allozyme electrophoresis was performed on adult male Tiger Swallowtail Butterflies in this study. Electrophoresis protocol follows that of Hagen and Scriber 1991. Adult specimens were removed from -80° C and processed in a 4° C cold room. Using a scalpel or razor blade, wings were dissected from the thorax at their place of attachment and returned to Glassine envelopes for previously discussed morphometric analysis. Tissue extracts were prepared by grinding one half of abdomen with 100 µl of an extraction buffer. The lower half of the abdomen was utilized in male specimens. The remaining abdomen portion, head and thorax, were returned to the -80° C freezer for future use. The extract was centrifuged for 10 minutes at 14,000 rpm. At this point the extract could be stored at -80° C until ready to continue the electrophoresis protocol. Female specimens were not utilized in the electrophoretic portions of this study for several reasons. First, the sample sizes for females were extremely low for many of the populations sampled. More importantly however, the allozyme banding patterns produced by females were frequently not as clear as those produced by male specimens

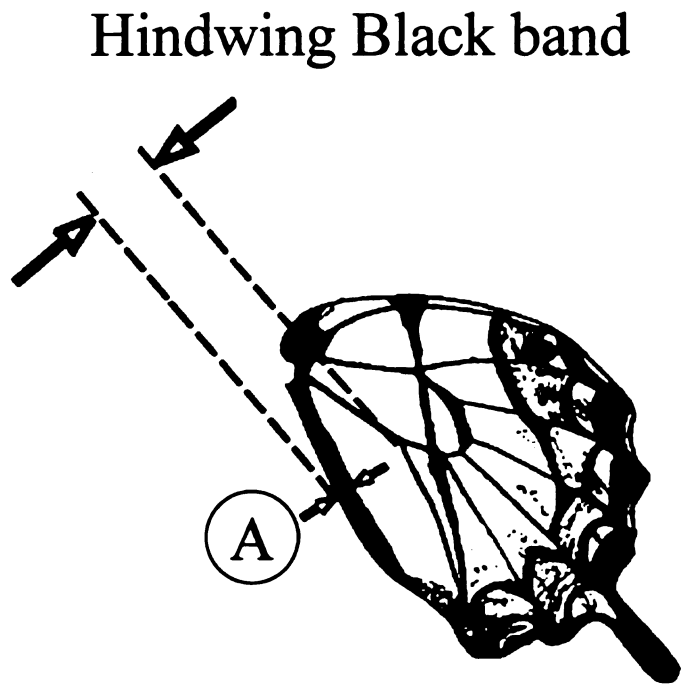


Figure 2.3. Black band width measurements are the percentage of the hindwing anal cell that is filled by the dark band labeled A. (Modified from Luebke et al. 1988)

and difficult to interpret. It is possible that the eggs contained in the abdomen somehow disrupt the normal staining process. When the electrophoresis process was performed on females, the upper portion of the abdomen was utilized so as to avoid possible sample contamination from spermatophores contained in the lower abdomen from previous matings.

Samples were removed from -80° C and allowed to thaw in 4° cold room for approximately 10 minutes and were then centrifuged for 5 minutes at 14,000 rpm. 7.5 µl of extract from each sample was applied to thin layer acetate plates (Titan III [94 by 76 mm], Helena Laboratories) for electrophoresis. The allozyme locus scored for this study portion is Pgd (6-Phosphogluconate dehydrogenase). Staining protocol and solution recipe is contained in Appendix A. Scoring of gel banding patterns was accomplished following methods of Hagen and Scriber 1991 using photographs or original gels and sketches.

Population Comparisons

Island populations, Isle Royale, Beaver, and South Manitou Islands, were each matched for comparative analysis with specimens from the most closely adjacent mainland populations available. Considerations were made related to distance from island to mainland location, comparable latitudes, and also direction for possible immigration due to prevailing winds. Isle Royale was compared to Cook county, Minnesota and Gogebic county, Michigan populations. Beaver Island was compared to Charlevoix and Dickinson county populations. South Manitou Island was compared to Charlevoix, Dickinson, and Mason county populations (Fig. 2.1).

Statistical Analysis

Multiple statistical analyses of both forewing length and black bandwidths were performed using JMP statistical software version 3.2 by Altura Software, Inc. One way anova was performed for all populations. The data sets for both wing measurements were evaluated for normality. In addition, black band percentage values were normalized using an arcsin transformation performed in Microsoft Excel 1997. Comparisons for all population pairs was accomplished using both Tukey-Kramer HSD and Student's t-test.

Statistical analyses were performed for population allele frequencies using the program Genepop v3.1 (Raymond and Roussett 1995). Tests for both genotypic and genic differentiation were performed for each island versus mainland comparison.

RESULTS

Wing Morphometrics

Two quantitative polygenic wing characteristics were chosen for analysis. Genetic differentiation between two populations is possible through phenotypic analysis because shared genes would be reflected in similar phenotypes (Boag & van Noordwijk 1987). The characteristics under investigation, forewing length and hind wing anal cell black bandwidth, were chosen for a combination of reasons. Both traits are heritable and polygenic (Luebke et al. 1988), thus conceivably impacted by mutation, genetic drift, and natural selection under differing local environmental conditions. In addition, these two traits were highly informative measurements to consider for indicating interspecific hybridization (Scriber et al. 2001), the later focus of this thesis.

Using both Tukey-Kramer HSD and Student's t-test at p-value of 0.05 (Table 2.1), *P. canadensis* forewing length measurements on Isle Royale have been shown not to be significantly different from those of Cook or Gogebic populations. Beaver Island showed no significant differences between either Charlevoix or Dickinson populations using Tukey-Kramer HSD, but did show a significant difference from the Dickinson population using the less rigorous Student's t-test (p-value < 0.01). Analysis using Tukey-Kramer HSD showed a significant difference for South Manitou Island from only the Dickinson population. Student's t-test indicated a significant difference between South Manitou Island and both Dickinson and Mason populations (p-values < 0.001 and 0.036 respectively).

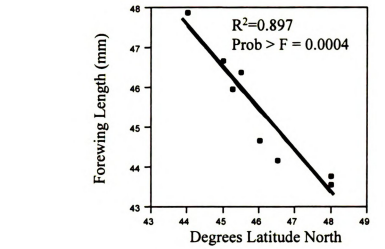
Table 2.1. 1998 Island versus mainland forewing length comparison. Island locations are printed in bold print with the corresponding comparative mainland populations following. Sample sizes and mean forewing length values ± standard deviation for each location are presented. t-test P-values for each island versus mainland pair wise comparison are listed below the corresponding mainland location. Values were computed using Microsoft Excel statistical analysis.

Location	**(n)**	**Mean ± Std. Dev.**	**t-test P-value**
Isle Royale	28	43.8 ± 2.1	
Cook Co.	35	43.6 ± 1.8	0.53
Gogebic Co.	34	44.2 ± 1.6	0.38
Beaver Island	11	46.4 ± 1.6	
Charlevoix Co.	50	46.0 ± 2.7	0.57
Dickinson Co.	63	44.7 ± 2.0	<0.01
South Manitou	32	46.7 ± 2.8	
Charlevoix Co.	50	46.0 ± 2.7	0.29
Dickinson Co.	63	44.7 ± 2.0	<0.001
Mason Co.	50	47.9 ± 2.3	0.036

Plotting the forewing length means for each population, in the order of decreasing latitude (Fig. 2.4), shows an apparent trend of increasing forewing length moving in a southerly direction. Plotting forewing length against latitude for each sampled population indicates a strong correlation between latitude and forewing length (R^2=0.897). This correlation has been shown to be generally true of populations of *Papilio* from Florida to Alaska (Scriber 1994).

Summaries of statistical analysis of hind wing black band widths using both Tukey-Kramer HSD and Student's t-test comparing island populations with mainland populations were completed using JMP statistical software. Summaries of t-test p-values are found in Table 2.2. The results are very comparable to those found for forewing length comparisons with only one exception. The Tukey-Kramer HSD analysis indicates that the Isle Royale population is not significantly different than those of Cook or Gogebic counties. In contrast, Tukey-Kramer HSD suggests that Beaver Island is not significantly different than either Charlevoix or Dickinson counties, where as the less rigorous Student's t-test indicates again that Beaver Island is significantly different from Dickinson county (p-value = 0.018). Both Tukey-Kramer and Student's t-test analysis agree that South Manitou Island black band widths are significantly different than those of both Charlevoix and Dickinson counties, but not from that of Mason county.

Again, comparable to that of forewing length, if mean black bandwidths for each population are listed in order of increasing latitude, a trend seems apparent and suggests that black bandwidth increases moving in a northerly direction (Fig. 2.5). If black



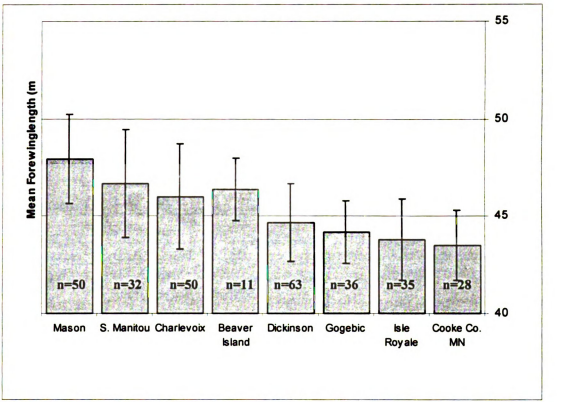


Figure 2.4. Correlation of population mean forewing length by increasing latitude. The populations sampled in 1998 are listed in order of increasing latitude (middle). Sample sizes are indicated in each histogram bar. The top figure represents the same populations arranged according to specific latitudes. The best linear fit is indicated, with R^2 value = 0.897 and Prob > F value = 0.0004. Statistical analysis was performed using JUMP Statistical software.

Table 2.2. 1998 Island versus mainland black band comparison. Island locations are printed in bold print with the corresponding comparative mainland populations following. Sample sizes and mean black band width percentage values ± standard deviation for each location are presented. t-test P-values for each island versus mainland pair wise comparison are listed following the corresponding mainland location. Values were computed using Microsoft Excel statistical analysis.

Location	**(n)**	**Mean ± Std. Dev.**	**t-test P-value**
Isle Royale	28	69.5 ± 4.9	
Cook Co.	35	71.4 ± 6.3	0.18
Gogebic Co.	34	67.6 ± 7.7	0.23
Beaver Island	11	60.8 ± 7.3	
Charlevoix Co.	50	62.4 ± 7.3	0.50
Dickinson Co.	63	67.0 ± 5.8	0.018
South Manitou	32	55.2 ± 7.3	
Charlevoix Co.	50	62.4 ± 7.3	<0.001
Dickinson Co.	63	67.0 ± 5.8	<0.001
Mason Co.	50	58.5 ± 7.7	0.057

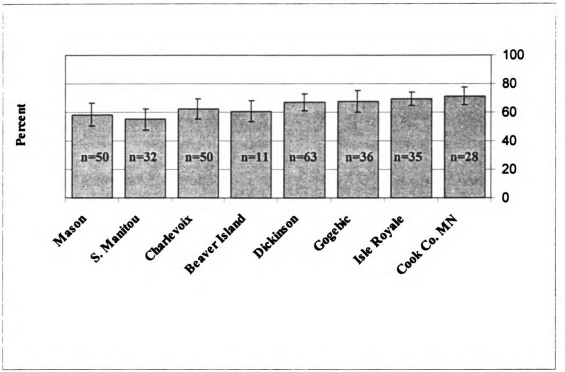


Figure 2.5. Mean black band width percentage and standard deviations for all populations sampled in 1998 listed in order of increasing latitude. Population sample sizes are indicated in histogram bars.

bandwidth is plotted against latitude for each sampled population (Fig. 2.6), a strong correlation between latitude and black bandwidth is evident (R^2=0.788). This too has been reported to generally be the case in populations of *Papilio* extending from Florida to Alaska. Overall, latitude may be playing a larger factor in black band differentiation for *P. canadensis* populations than does any island effect, with the exception of possibly South Manitou Island.

Allozyme Electrophoresis

Of the nine Pgd alleles that exist in the closely related species groups of Papilionidae (Hagen & Scriber 1991), six were encountered in this thesis analysis. In addition, one undescribed rare allele was encountered in one of the study populations. Population allele frequencies are listed in Table 2.3. Two statistical analyses were performed to identify genetic differentiation between island and mainland populations: 1)genotypic differentiation tests whether the distribution of genotypes is identical between population pairs; 2)genic differentiation tests whether the distribution of alleles is identical between population pairs.

Analysis of genotypic differentiation indicates that both Isle Royale and Beaver Island are not significantly different than their respective mainland counterparts. South Manitou Island however, has been shown to be significantly different from all of its mainland comparison populations, Charlevoix, Dickinson, and Mason counties with p-values of 0.00289, 0.00002, and 0.00728 respectively (Table 2.4). Analysis of genic differentiation for each of the study populations directly correlates to the genotypic differentiation, with Isle Royale and Beaver Island not being significantly different from

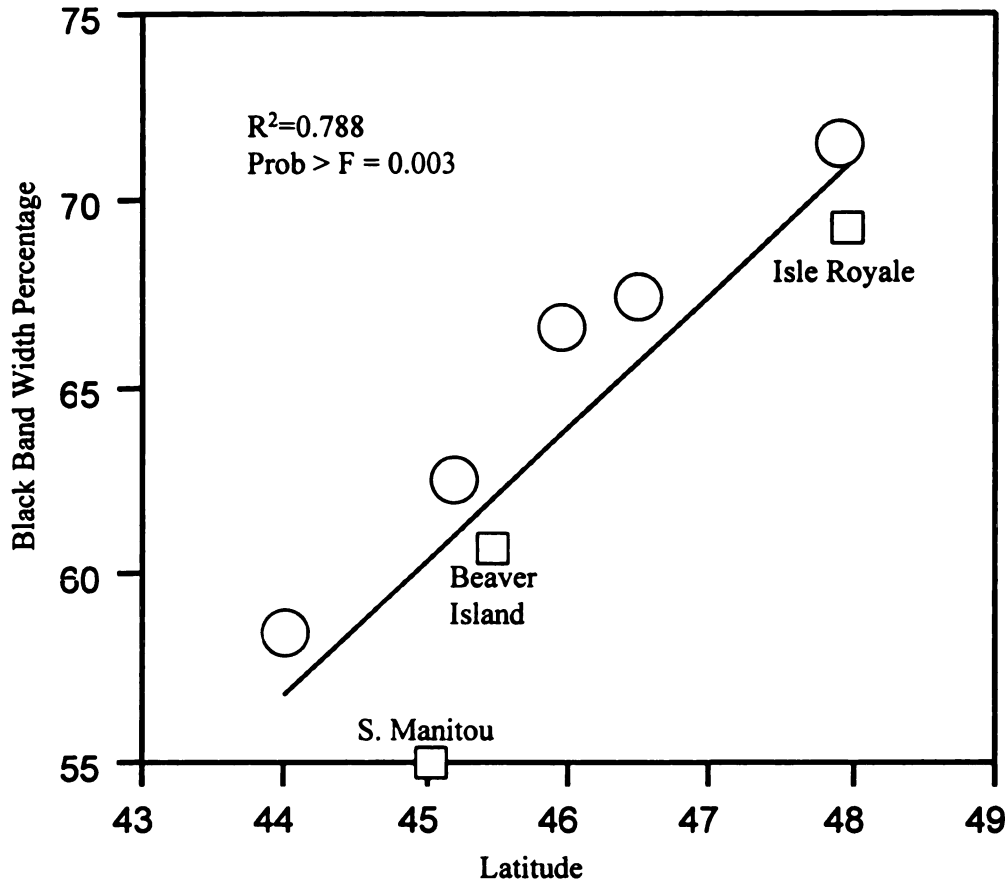


Figure 2.6. Correlation of black band width versus latitude. Circles represent mainland populations while squares represent island populations. Rsquare value = 0.788 and Prob > F value = 0.003.

Table 2.3. 1998 population Pgd allele frequencies. Each sampled population is listed with the sample size in parentheses below. Island populations are printed in bold type. Alleles marked with an asterisk indicate a *Papilio glaucus* type allele.

	Allele						
Population	-150	-137	-125	*-100	-90	-80	*-50
Isle Royale (28)	0.018	0	0.893	0	0	0.089	0
Dickinson (48)	0.069	0	0,828	0	0.009	0.095	0
Gogebic (36)	0	0	0.863	0	0	0.137	0
Cook (35)	0.029	0	0.886	0	0	0.086	0
Beaver Island (11)	0	0	1.00	0	0	0	0
Charlevoix (50)	0.026	0	0.906	0.009	0	0.051	0.009
South Manitou (32)	0	0	0.875	0.109	0	0.016	0
Mason (50)	0.035	0.009	0.878	0.009	0	0.07	0

Table 2.4. Genotypic differentiation for each island and its adjacent mainland populations at the PGD locus. H_0: the genotypic distribution is identical between population pairs. Statistical analysis performed in Genepop Version 3.1d 1999. Population pairs with P-values of significance <0.05 are in bold print.

Populations Compared	**P-value ± S.E.**
Isle Royale & Cook	1.0000 ± 0.0000
Dickinson	0.3678 ± 0.0063
Gogebic	0.3073 ± 0.0046
Beaver Island & Charlevoix	0.4321 ± 0.0043
Dickinson	0.0556 ± 0.0031
South Manitou & Charlevoix	**0.0029 ± 0.0004**
Dickinson	**0.00002 ± 0.00001**
Mason	**0.0073 ± 0.0008**

their mainland counterparts and South Manitou Island again being significantly different from all of its mainland comparison populations (Table 2.5).

Table 2.5. Genic (allelic) differentiation for each island and its adjacent mainland population at the PGD locus. H_0: the allelic distribution is identical between population pairs. Statistical analysis performed in Genepop Version 3.1d 1999. Population pairs with P-values of significance <0.05 are in bold print.

Populations	**P-value ± S.E.**
Isle Royale & Cooke	1.0000 ± 0.0000
Dickinson	0.5641 ± 0.0061
Gogebic	0.4057 ± 0.0056
Beaver Island & Charlevoix	0.8298 ± 0.0044
Dickinson	0.2964 ± 0.0053
South Manitou & Charlevoix	**0.0054 ± 0.0009**
Dickinson	**0.0000 ± 0.0000**
Mason	**0.0026 ± 0.0005**

DISCUSSION

Based upon the combined analyses performed, I found little evidence to suggest that the *Papilio canadensis* populations living on either Isle Royale or Beaver Island have genetically differentiated from their mainland counterparts. These findings are consistent with the results of other investigations on gene flow in various other *Papilio* species (Tong & Shapiro 1989, Bossart & Scriber 1995, Aubert et al. 1997, Hoole et al. 1999). In addition, these findings further support the conclusion that there is little genetic structuring in Great Lakes area *P. canadensis* populations (Stump 2000). South Manitou Island however, exhibits significant differences from mainland counterpart populations for all analyses performed. This suggests that there is significant genetic differentiation between *P. canadensis* on South Manitou Island from the surrounding mainland populations.

The analyses performed comparing the Isle Royale population and the mainland counterpart populations of *P. canadensis* indicate that there is no significant difference for any of the characters under scrutiny. Isle Royale appears extremely similar to its nearest mainland counter part, Cook County, Minnesota, for all characteristics. The most powerful technique applied to these populations being allozyme electrophoresis indicates that these two populations are nearly identical in allele frequencies. Intuitively, of the two mainland populations compared to Isle Royale, Cook County is the most likely location for gene flow to and from. These analyses would indicate that there is sufficient gene flow between Isle Royale and the adjacent mainland to prevent any genetic differentiation.

The Beaver Island population of *P. canadensis* is also very similar to its mainland counterparts for each of the analyses performed. Beaver Island however differs significantly from the Dickinson County population for wing morphometrics. Both forewing length and black bandwidth have been shown to be significantly different ($p < 0.001$ and $p=0.018$ respectively) between these two populations. Beaver Island is not significantly different from its more adjacent mainland counterpart population, Charlevoix County, for these wing characteristics. This lack of consistent differences between its mainland comparison populations suggests that any differentiation might not be due to an island effect and might better be explained using an alternative hypothesis.

The striking feature of the *P. canadensis* assayed from the Beaver Island population is the allele frequencies represented (Table 2.3). The Beaver Island samples show the population being fixed at a single allele (-125) for the Pgd locus. This is in great contrast to both of the mainland comparison populations that exhibit -150, -125, -100, -90, -80, and -50. Dickinson and Charlevoix counties are both represented by the largest allele diversity of any of the populations under scrutiny. It has been suggested that a common phenomenon in isolated populations of finite effective size, is a marked decrease of heterozygosity (Carlquist 1974, Jacquard 1974, Hartl 1988, Grant 1998). However, the sample size taken from Beaver Island is relatively small. In fact it is the smallest sample size in this study. Perhaps an increased sample size would show this apparent homozygosity as being an artifact of small sample size.

The population of *P. canadensis* on South Manitou Island is the most intriguing portion of this island study. This island population has been shown to be significantly different from all of the mainland populations it has been compared to. Early on in this investigation, allozyme electrophoresis on the Pgd locus suggested that there was a high level of genetic introgression, from a closely related southern species, *P. glaucus*. The Pgd -100 allele is diagnostic in distinguishing between *P. canadensis* and *P. glaucus* (Hagen et al. 1991). This allele has been shown to occur at relatively high frequencies in the South Manitou Island population (Table 2.3). High levels of introgression from *P. glaucus* into this *canadensis* population would help explain the observed island versus mainland differences for each of the characters studied. Further discussion and investigation of this introgression on South Manitou Island and the adjacent populations are the focus of Chapter 3.

CHAPTER 3:

ISOLATED "HYBRID SWARM": INTROGRESSED GENES OF *PAPILIO GLAUCUS* IN A *P. CANADENSIS* POPULATION FAR BEYOND THEIR HYBRID ZONE

Introduction

Introgression is the process by which alleles are exchanged from one species into the gene pool of another. Introgression occurs as a result of hybridization. Hybridization is the production of offspring by parents from populations that are diagnosably distinct for one or more characters. A hybrid is an individual that is heterozygous (intermediate) for any one or more of these characters. A "hybrid swarm" is a localized area of individuals exhibiting a diverse array of recombinant types (Harrison 1993).

It has been generally assumed that hybrids are unfit relative to parental types, and that they are evolutionary dead ends. However, recently this view has been challenged (Arnold & Hodges 1995). Hybrid vigor has been observed for certain traits in lab reared crosses of *Papilio glaucus* and *P. canadensis* (Scriber et al. 2001). It is believed that hybrid zones are maintained by a balance of selection and dispersal (Porter et al. 1997).

Papilio glaucus and *P. canadensis* are closely related butterflies, but are distinct species (Hagen et al. 1991). Several diagnostic characteristics (morphologic, ecological, physiological, and biochemical) are extremely useful in distinguishing between these two species (Table 3.1; Scriber 1990). Where the ranges of these two butterflies meet, a very narrow zone of hybridization occurs. In Michigan this hybrid zone is between 43° and

Table 3.1. Summary of selected species differences discussed between *Papilio glaucus* and *P. canadensis.* (Modified from Table 1 in Scriber 1990).

Characteristic	*glaucus*	*canadensis*
(Morphological)		
Adult size (forewing length)	Long	Short
Hindwing anal cell black band	Narrow	Wide
(Ecological/Physiological)		
Tulip tree oviposition preference	Yes	No
Quaking aspen oviposition preference	No	Yes
Tulip tree detoxification ability	High	Low
Quaking aspen detoxification ability	Low	High
(Biochemical)		
Pgd (X-linked) allozymes	PGD -50, -100	PGD -80, -125
Ldh (X-linked allozymes	LDH 100	LDH 40, 80
Hk (autosomal) allozymes	HK 100	HK 110

44° latitude and has been stable for at least two decades (Scriber 1982; Scriber et al. 1996; Figure 3.1).

Papilio glaucus and *P. canadensis* introgression has been documented for sex-linked and autosomal diagnostic allozyme loci (Pgd, Ldh, and Hk) (Hagen et al. 1991). Introgression of *P. canadensis* into populations of *P. glaucus* has been suggested as a possible explanation of the "spring form" of *Papilio glaucus* described throughout Eastern North America (Scriber 1990). More recently, introgression of *P. glaucus* mtDNA has been described in populations of *P. canadensis* (Stump 2000). However, the majority of this introgressed hybridization between *P. canadensis* and *P. glaucus* described has been noted in locations close to the described hybrid zone (e.g. Isabella County and Mason County).

Chapter 2 indicated that the population of *Papilio canadensis* found on South Manitou Island exhibits significant differences, both morphological and biochemical, from the surrounding mainland populations of *P. canadensis.* Early on in this investigation it was realized that the *P. canadensis* population on South Manitou Island exhibited *glaucus*-like traits. South Manitou Island is approximately 150 kilometers north of the *canadensis / glaucus* hybrid zone in Michigan. High levels of introgression have not yet been described at such a great distance from the *canadensis / glaucus* hybrid zone.

The extent to which *P. glaucus* introgression was present on and around South Manitou Island was investigated through analysis of several diagnostic characters between *P. canadensis* and *P. glaucus.* A combination of morphologic, ecological and

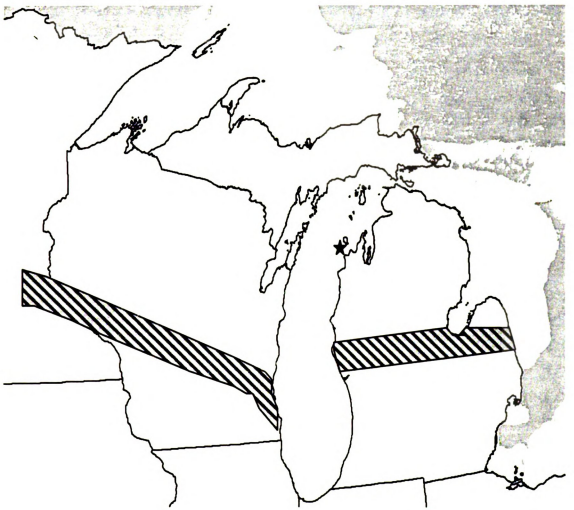


Figure 3.1. The shaded region in Michigan roughly indicates what has historically been considered the hybrid zone between *Papilio glaucus* and *P. canadensis* (Nielsen, 1999). The shaded region across Wisconsin into Minnesota represents the 50 year average degree day accumulation and northern limit allowing for two generations of *Papilio glaucus*. The star indicates the location of South Manitou Island.

biochemical traits were considered using samples taken over a three-year period (1998-2000).

MATERIALS AND METHODS

Specimen Acquisition and Transport

Techniques for *Papilio* collection and processing follow those used in Chapter 2. Additional specimen collection sites and sample sizes are listed in Table 3.2.

Specimen Processing

Male specimens were preserved by freezing them alive in an -80° C ultra-low biological freezer. Male specimens that were to be utilized for laboratory hand-pairings were maintained in a 4° C refrigerator, and frozen (-80° C) alive at a later time. In order to extend their mating potential and vigor, these were fed a solution of honey water, sodium, and amino acids every other day. This was accomplished by extending their proboscis with a straightened paper clip. The tip of the proboscis was then placed into a teaspoon of the 20% honey/water/mineral solution held in a plastic spoon. Individuals would readily feed for up to 15 minutes. Feeding individuals were left under a small screen cage until they were finished drinking and left the feeding station, after which time they were placed back into the Glassine envelopes and returned to the refrigerator. Prior to and after feeding, these individuals were given approximately 30 minutes to acclimate to room temperature and to digest their meals. After utilization for hand pairing, these males were also frozen alive at -80° C.

Upon arrival, female specimens were processed by recording their capture date and location, and given a "Mother Number ID" by which we could later label and identify that individual's offspring. Female specimens were fed daily, but on a 20%

Table 3.2. Additional sampling sites from 1998-2000. Male and female sample sizes and dates of collection are provided for each sample location.

	Location	Males	Females	Dates collected
1998	Oscoda Co., Michigan	13	0	17 May
	Isabella Co., Michigan	50	13	18-24 May
	Lawrence Co., Ohio	22	0	14 May
1999	Emmet Co., Michigan	24	4	30 May, 8 June
	Charlevoix Co., Michigan	8	0	8 June
	South Manitou Island	120	52	18-19 June
	Benzie Co., Michigan	7	0	27 May
2000	South Manitou Island	100	51	10-11, 26-27 June
	North Manitou Island	96	1	9 June
	Leelenau Co., mainland	68	8	3 June
	Charlevoix Co., Michigan	33	0	3 June
	Benzie Co., Michigan	11	1	3 June
	Oscoda Co., Michigan	21	0	9 June
	Mason Co., Michigan	17	8	2 June
	Isabella Co., Michigan	14	0	4 June

honey water solution. Females were kept alive and utilized for oviposition preference assessment for sometimes up to 10 days. When female specimens were too weak to hold onto an extended finger or immediately after their deaths, they too were preserved at -80° C.

Wing Morphometrics

Two wing characteristics were chosen for analysis in order to identify the presence of *Papilio glaucus* introgression into the South Manitou Island population of *P. canadensis.* Forewing length and hind wing anal cell black bandwidth are morphometric characters of adults which can be used in the field to help distinguish between *P. canadensis* and *P. glaucus*. On the average, *P. glaucus* forewings are significantly larger than those of *P. canadensis*. *P. glaucus* forewings have been shown to be 8-10 mm longer, from thoracic attachment to tip, than *P. canadensis* forewings (Hagen et al 1991). The more powerful diagnostic morphometric wing character is the width of the black band along the anal margin of the hindwing. For *P. glaucus* males, this band fills 10 to 50 percent of the width from the wing margin to the CuA2 vein; whereas for *P. canadensis* the width of the band fills 50 to 90 percent of this anal cell (Hagen et al. 1991). Hybrid individuals display a black bandwidth intermediate between the two, averaging 50 percent (Scriber et al. 2001). Intermediacy for genetically based morphometric traits is a good indicator of a hybrid individuals or populations (Harrison 1993).

The two wing traits were compared for all males captured from South Manitou Island, a pure *canadensis* population in Oscoda County, and a pure *glaucus* population

from Lawrence county, Ohio. Methods used to score and statistically analyze these two traits follow that used in Chapter 2.

Oviposition Host Preference Assessment

South Manitou Island female oviposition host preferences, for 1998-2000, were assessed in a 3-choice arena (see Scriber 1993). Individual females (1998 n=24; 1999 n=52; 2000 n=50) were placed in clear polystyrene circular ventilated dishes, 10" in diameter and 4" in depth. The bottom of the dish was lined with a sheet of Acclaim "Natural" paper toweling. The dish contained leaves of three natural Tiger Swallowtail butterfly host plants [Quaking Aspen (*Populus tremuloides*), Tulip tree (*Liriodendron tulipifera*), and Black Cherry (*Prunus serotina*)], of approximately the same quantity, equally spaced and randomly placed around the outside edge of the arena. Host choices were kept turgid in the arenas by supporting the petioles in rubber-capped plastic florist aquapics containing water. Arenas were placed on turntables that rotated the dishes approximately once every 5 minutes. These turntables were situated with 100 watt incandescent lamps, approximately 0.5 meters away from one side, that were on a timed photoperiod of six hours on, six hours off. This arena set up was housed in a room with no natural lighting. While rotating, the females would be attracted to the side of the arena that the light was then shining upon. She would there encounter each of the three host plants in turn.

Approximately the same time each day, females were removed and fed. At this time eggs that had been laid since the previous day were collected and recorded. Only eggs that were directly laid upon one of the three host plant choices were recorded as

such. Eggs that were laid on the sides of the dish and/or the paper towel were recorded as "other". Many of these were within "reach" of the female abdomen while forelegs were touching the leaf. Eggs were removed from the arena by cutting away the leaf surface or paper towel that they were attached to using small scissors. Those attached to the sides of the plastic dish were gently removed with the tip of a finger, after first loosening them with a bit of fresh water expelled from a water bottle. At this time, host plants were replaced as needed. Collected eggs were placed in appropriately labeled 150mm diameter Lab-Tek® polystyrene petri dishes lined with paper towel. These were then incubated in Percival® Growth Chambers at 23° C (18:6 photo/scoto-phase) and monitored daily for hatching.

Host plants were collected from various sites near Michigan State University campus in Ingham County, approximately every other day. Only the most vigorous and least blemished leaves available were collected. In the lab, cut host plants were maintained in a bucket of water, covered in large black plastic bags, and kept in a dark cold (4° - 6° C) storage unit to prevent desiccation.

Oviposition "preference" was only assigned to females laying > 10 eggs total, and > 50% of these eggs were laid on the same host plant.

Larval Survivorship

1999 and 2000 larvae were assayed for differential first instar survivorship on the three host plants. Incubating eggs were monitored daily for hatching. Eggs hatched after approximately 3 – 6 days. As eggs hatched, the neonate larvae were equally and sequentially distributed on each of the three host plants, Black cherry, Quaking aspen,

and Tulip tree (1999 n=146 eggs; 2000 n=736 eggs). First instar larvae were carefully picked up using the moistened fine tip of a camel hair paint brush. This was accomplished using a gentle rolling motion of the brush tip. Larvae were then placed on fresh host plants in appropriately labeled 150mm petri dishes lined with paper towel. No more than 6 first instar larvae were placed in the same petri dish. Host plants were kept turgid and fresh by placing them in water filled aquapics. Larvae were checked for survival after 5-6 days for each petri dish, which generally included the duration of the first instar.

Survival was analyzed in two different ways. The percent of first instar larvae from each individual family surviving on each host plant was recorded, and then the mean family percent survival was calculated across all families. In addition, the total number of first instar larvae surviving on each host was recorded in order to calculate the total percent survival across the population. For *P. canadensis* it was expected to have high first instar survivorship on both Quaking aspen and Black cherry. *P. canadensis* does not however have a strong ability to detoxify Tulip tree. This being the case, any *P. canadensis* survival on Tulip tree was considered to be significant.

Allozyme Electrophoresis

Allozyme electrophoresis was performed following the same techniques and analysis outlined in Chapter 2. Analysis was performed for male samples from 1998, 1999, and 2000. In 1998 electrophoresis was performed for the diagnostic Pgd allozyme for all populations. Additional diagnostic allozyme loci (Ldh and Hk) were assayed for South Manitou Island, Isle Royale, Beaver Island, and Oscoda County in 1998. Male

specimens collected from all populations in 1999 were assayed for three species diagnostic allozyme loci. Male specimens collected from all populations in 2000 were assayed only for Pgd and Hk loci. After previous analysis of the Ldh locus for hundreds of specimens that indicated absolutely no deviation from the expected *canadensis* allele, it was decided to not spend valuable time and resources on further population wide analysis of this locus. To be sure that no primary hybrids were present in any population, only specimens that scored *glaucus*-like for Pgd were also analyzed and scored for the Ldh locus. Allele frequencies are reported as percentages of *canadensis* vs. *glaucus* alleles for each allozyme locus encountered in the populations described.

RESULTS

Wing Morphometrics

Forewing length for South Manitou Island (mean = 46.7 ± 0.49 s.e.) did not significantly differ from that of the *canadensis* population in Oscoda County (mean = 46.0 ± 0.92 s.e.) but did differ significantly from that of the *glaucus* population from Lawrence county, Ohio (mean = 49.0 ± 0.53 s.e.) using both Tukey-Kramer and Student's t test (Figure 3.2). South Manitou Island black bandwidth (mean = 55.2 % ± 1.29 s.e.) was found to be significantly different from those of both Oscoda (mean = 63.4 % ± 1.71 s.e.) and Lawrence (mean = 35.5 % ± 1.94 s.e.) counties (Figure 3.3).

Oviposition Preference

The results for oviposition preference for 1998-2000 are presented in tables 3.3, 3.4, and figure 3.4. In 1998, of 24 South Manitou Island females assayed, eight females laid >10 eggs in 3-choice arenas. Of these eight, only five displayed >50% preference for any single available host plant option. Of these five, four females exhibited oviposition preference for Tulip tree. From all 24 females assayed, 301 eggs were oviposited in total. The greatest portion of this total (56%) was oviposited on Tulip tree. The next most frequently chosen host plant was Quaking aspen (20%).

In 1999, of the 52 South Manitou Island females assayed, 18 females oviposited >10 eggs. Of these 18, 15 displayed >50% preference for any single available host plant option. nine of these 15 females preferred to oviposit on Quaking aspen, while six preferred Tulip tree. From the 52 females assayed, 1161 eggs were oviposited in total.

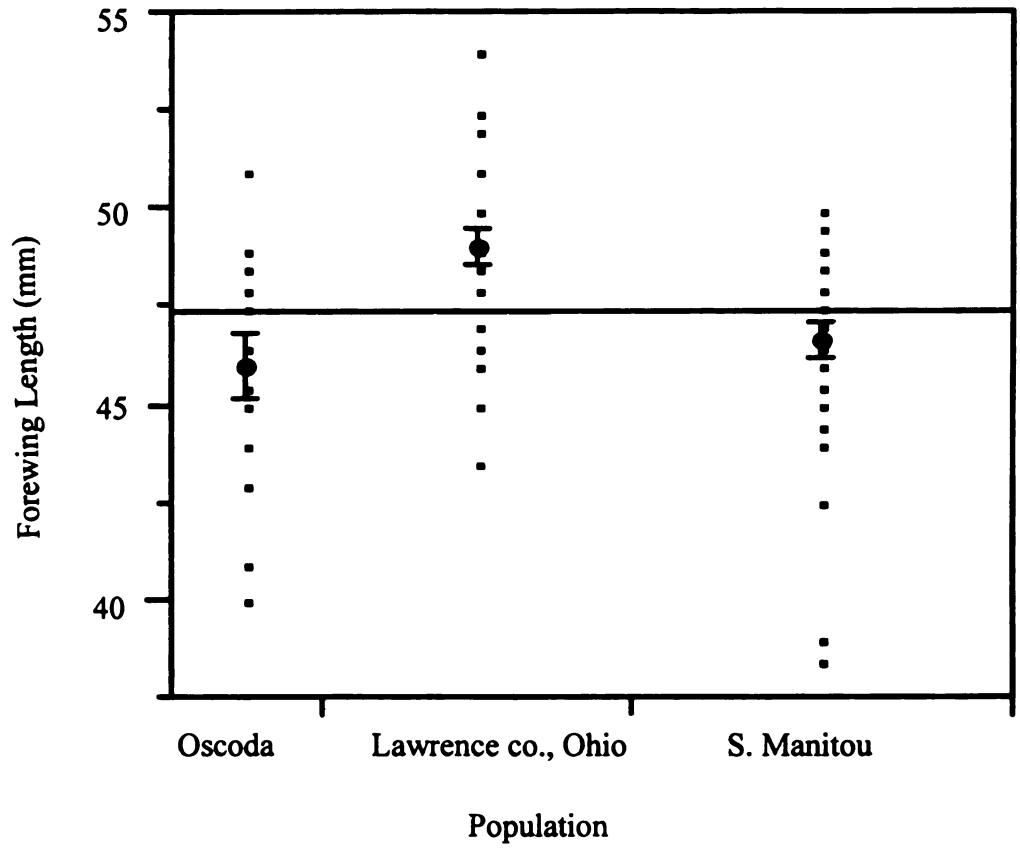


Figure 3.2. Forewing length comparison between *P. canadensis* from South Manitou Island, Oscoda co., and a population of *P. glaucus* from Lawrence co., Ohio. Mean forewing length for each population is shown with error bars indicate ± s.e. (S. Manitou = 46.7 ± 0.49, Oscoda co. = 46.0 ± 0.92, *P.g.* Ohio = 49.0 ± 0.53). Comparison using both Tukey-Kramer and Student's t-test at alpha value = 0.05 indicates that there is no significant differences between S. Manitou and Oscoda co. while there is a significant difference between S. Manitou and Lawrence co., Ohio (p-value = 0.003).

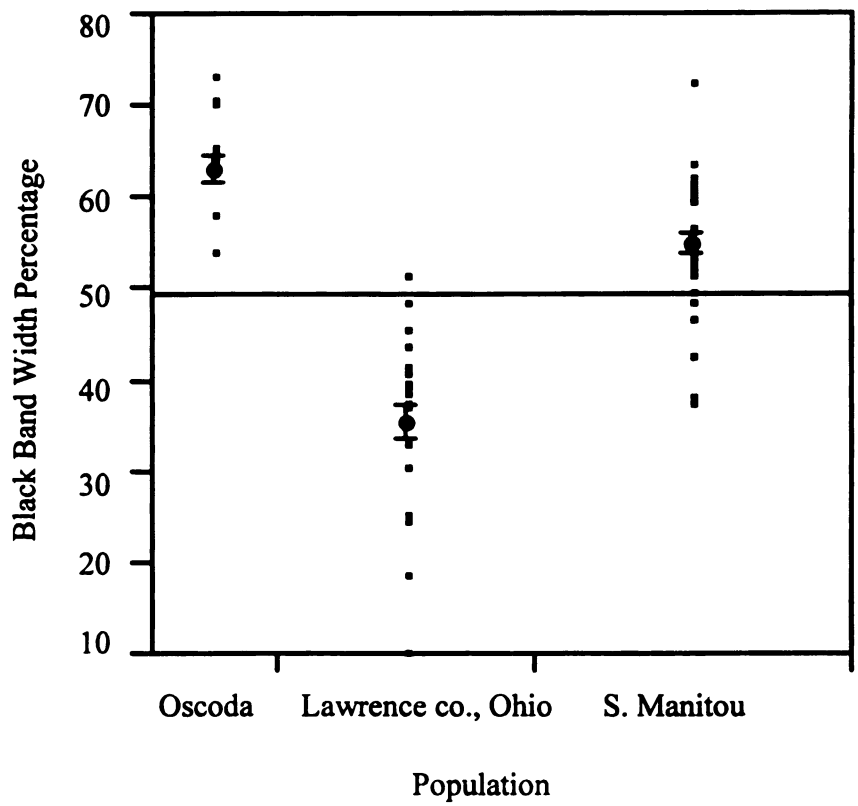


Figure 3.3. Black band width comparison between *P. canadensis* from South Manitou Island, Oscoda co., and a population of *P. glaucus* from Lawrence co., Ohio. Mean black band widths for each population are shown with error bars indicating ± s.e. (S. Manitou = 55.20 ± 1.29, Oscoda co. = 63.38 ± 1.71, *P.g.* Ohio = 35.50 ± 1.94). Comparison using both Tukey-Kramer and Student's t-test at alpha value = 0.05 indicates that South Manitou differs significantly from both Lawrence co. and Oscoda co. (p-values < 0.001).

Table 3.3. South Manitou 3-choice oviposition preference for individual female 1998-2000. Only females ovipositing >10 eggs were used for this analysis. The total number of eggs oviposited by each female is indicated as well as the percentage of the eggs placed on each host plant. Percentages >50% are in bold print indicating that that female expressed a "preference" for that particular host plant. The other category includes many eggs that were placed within 2 cm of the host plant and may have been intended for that host. For the purposes of this study they have been scored as *other*.

		Percent of eggs per host			
1998	**(n) # of eggs laid**	Black cherry	Tulip tree	Quaking aspen	* Other
Mother ID					
14220	13	0	23	**62**	15
14222	16	0	**75**	13	12
14223	36	25	47	28	0
14225	28	0	**82**	18	0
14226	46	28	41	28	3
14229	60	15	**73**	12	0
14230	71	13	**55**	18	10
14239	10	30	20	40	10
	Mean ± s.e.	13.9 ± 4.6	52.0 ± 8.3	27.4 ± 5.9	6.3 ± 2.2

		Percent of eggs per host			
1999	**(n) # of eggs laid**	Black cherry	Tulip tree	Quaking aspen	* Other
Mother ID					
15227	98	13	**61**	17	8
15228	23	0	4	**96**	0
15234	13	0	**69**	8	23
15239	49	2	**76**	2	20
15242	22	0	9	**82**	9
15243	51	4	27	**59**	10
15250	10	0	10	**70**	20
15251	54	6	**52**	15	28
15253	117	18	40	21	21
15254	117	3	17	38	42
15261	103	6	**81**	4	10
15264	118	4	6	**77**	13
15265	14	7	14	**50**	21
15266	110	1	7	**84**	7
15267	69	0	39	39	22
15269	21	29	0	**52**	19
15272	62	15	**73**	5	8
15273	51	8	14	**61**	18
	Mean ± s.e.	6.4 ± 1.8	33.3 ± 6.7	43.3 ± 7.3	16.6 ± 2.3

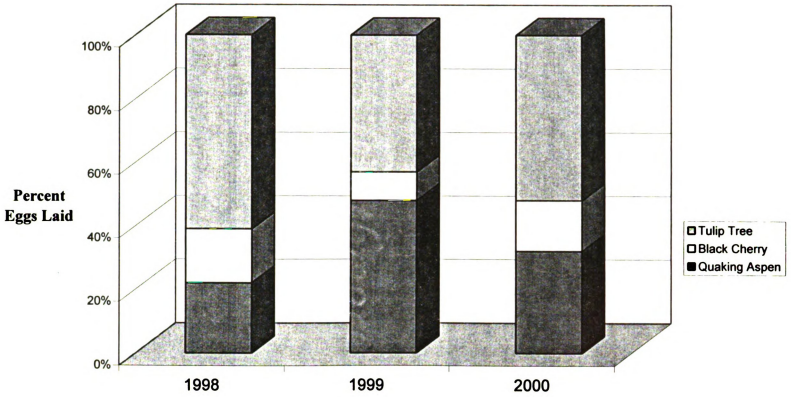
Table 3.3 continued

2000	(n) # of eggs laid	Percent of eggs per host			
		Black cherry	Tulip tree	Quaking aspen	* Other
Mother ID					
16136	56	0	**50**	32	18
16137	81	20	**63**	6	23
16175	21	19	**71**	5	5
16177	90	7	**59**	11	23
16178	79	19	**54**	14	13
16181	14	7	7	36	50
16183	61	11	10	61	18
16185	36	11	17	44	28
16190	41	5	41	20	34
16191	131	2	7	45	47
16192	100	19	39	12	30
16197	34	3	**65**	18	15
16198	64	28	41	25	6
16199	53	28	25	26	21
16202	190	14	**59**	13	14
16215	11	9	0	**73**	18
16219	58	3	34	41	21
	Mean ± s.e.	12.1 ± 2.1	37.8 ± 5.6	28.4 ± 4.7	22.6 ± 3.0

Table 3.4. South Manitou population total 3-choice oviposition preference 1998-2000. The eggs of all females that laid any eggs (>10 and <10) were pooled to provide an indication of the total population oviposition preference. Total number of eggs laid by all females is provided as well as the number of females that were assayed.

		Percent of eggs per host			
(n) # of eggs laid		Black cherry	Tulip tree	Quaking aspen	Other
1998 Population totals n=24 females	301	16%	56%	20%	8%
1999 Population totals n=52 females	1161	7%	36%	40%	17%
2000 Population totals n=50 females	1120	12%	41%	25%	22%

Figure 3.4. South Manitou population total 3-choice oviposition preference.



1998	1999	2000
n=24 females	n=52 females	n= 50 females
n=16 females laid eggs	n=34 females laid eggs	n=23 females laid eggs
n=301 eggs	n=1161 eggs	n=1120 eggs

The greatest portion of this total was oviposited on Quaking aspen (40%), closely followed by Tulip tree (36%) with the rest on Black Cherry (7%) or "other" (17%).

In 2000, of the 50 females assayed, 17 females oviposited >10 eggs. Of these 17, only eight exhibited >50% host preference. Of these eight females, seven displayed an oviposition preference for Tulip tree. From the 50 females assayed in 2000, 1120 eggs were oviposited total. Of this total, the greatest portion was placed on Tulip tree (41%) followed by Quaking aspen (25%).

Larval Survivorship

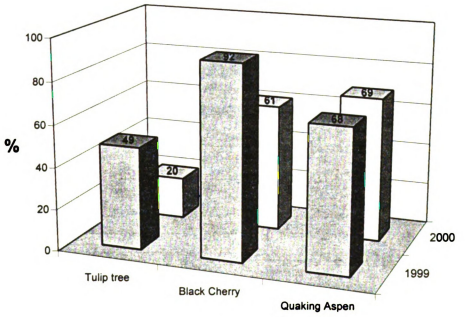
In 1999, of the 146 larvae assayed for detoxification abilities across the three host plant options, 52% of those placed on Tulip tree survived past the first instar, while 82% and 75% survived on Black cherry and Quaking aspen respectively (Fig. 3.5). When considering the average survival of all females, the mean family percent survival was as follows: Tulip tree – 49%; Black Cherry – 82%; Quaking aspen – 68%. In 2000, of the 736 larvae assayed, 18% of those placed on Tulip tree survived, while 58% and 65% survived on Black cherry and Quaking aspen respectively. Mean family percent survival rates were Tulip tree – 20%; Black cherry – 61%; Quaking aspen 69%.

Allozyme Electrophoresis

In 1998, the South Manitou Island population of *Papilio canadensis* exhibited unusually high levels of *glaucus* alleles at the Pgd locus (10.9%). This is markedly higher than any other Michigan population sampled in 1998; Charlevoix (1.8%), Mason (0.9%), and Isabella counties (4.0%). The South Manitou Island population also exhibited significant levels of *glaucus* Hk allele introgression (4.7%). Of the populations

Figure 3.5. South Manitou larval host plant survival 1999-2000. Approximately equal numbers of the eggs from each female that laid >10 larvae were distributed on each of the host plant options (Tulip tree, Black cherry, and Quaking aspen). The mean family % survival is the average percentage of larvae that survived through the first instar on each host plant, where as the total % survival is the percentage of all assayed larvae that survived on each host plant.

Year	Host Plant	(n) larvae	Total % Survival	(n) # of families	Mean Family % Survival
1999	Tulip tree	50	52	10	48.8±10.7 s.e.
	Black Cherry	56	82	10	92.2± 3.8 s.e.
	Quaking Aspen	40	75	7	67.5±13.1 s.e.
2000	Tulip tree	255	18	18	20.2± 5.3 s.e.
	Black Cherry	232	58	18	58.3± 7.5 s.e.
	Quaking Aspen	249	65	18	68.7± 6.2 s.e.



assayed for Hk, South Manitou was the only population that showed any levels of introgression at this locus (Table 3.5, Fig. 3.6). No populations assayed for Ldh, including South Manitou Island, showed any level of *glaucus* type alleles for this locus.

The 1999 samples assayed appeared similar in allele frequencies to those in 1998, with South Manitou Island exhibiting 7.9% *glaucus* alleles for Pgd (Table 3.5, Fig. 3.7). In addition, the adjacent mainland population of *P. canadensis* of Leelenau County exhibited unusually high levels of *glaucus* alleles (5.2%). None of the other surrounding counties sampled and assayed had any *glaucus* alleles for the Pgd locus. There were however, low levels of *glaucus* alleles found for the Hk locus in most of the populations surveyed: South Manitou (5.4%); Leelenau (3.4%); Emmet (4.0%); Benzie (7.1%); Charlevoix (0.0%). Again in 1999, there were no *glaucus* alleles (LDH 100) found for the Ldh locus in any population assayed including South Manitou Island.

The 2000 samples (from a wider geographic area) showed populations exhibiting *glaucus* alleles for both Pgd and Hk (Table 3.5, Fig. 3.8). South Manitou Island continued to have the highest levels of *glaucus* Pgd alleles (9.5%). Again in 2000, for all populations assayed, there were no *glaucus* alleles found for the Ldh locus.

Table 3.5. Allele frequencies for three diagnostic loci present in each of the study populations from 1998-2000. Values presented are percentages of *Canadensis* versus *glaucus* alleles detected. Dashes indicate that electrophoresis was not performed for that locus for that population.

1998		**Allele** (*canadensis*) (*glaucus*)	
Population (n)	**Pgd**	**Ldh**	**Hk**
Cook Co., MN	1.00	----	----
(35)	.0	----	----
Isle Royale	1.00	1.00	1.00
(28)	.0	.0	.0
Gogebic Co.	1.00	----	----
(36)	.0	----	----
Dickinson Co.	1.00	----	----
(48)	.0	----	----
Beaver Island	1.00	1.00	1.00
(11)	.0	.0	.0
Charlevoix Co.	0.983	----	----
(50)	0.018	----	----
South Manitou	0.891	1.00	0.953
(32)	0.109	.0	0.047
Isabella Co.	0.96	----	----
(50)	0.04	----	----
Oscoda Co.	1.00	1.00	1.00
(13)	.0	.0	.0
Mason Co.	0.992	----	----
(50)	0.009	----	----

Table 3.5 continued.

1999		**Allele** (*canadensis*) (*glaucus*)	
Population (n)	**Pgd**	**Ldh**	**Hk**
Emmet Co.	1.00	1.00	0.96
(25)	.0	.0	0.04
Charlevoix Co.	1.00	1.00	1.00
(8)	.0	.0	.0
South Manitou	0.921	1.00	0.946
(120)	0.079	.0	0.054
Leelenau Co.	0.948	1.00	0.966
(29)	0.052	.0	0.034
Benzie Co.	1.00	1.00	0.929
(7)	.0	.0	0.071

Table 3.5 continued.

2000		**Allele** (*canadensis*) (*glaucus*)
Population (n)	**Pgd**	**Hk**
North Manitou Island	0.937	0.932
(96)	0.063	0.068
South Manitou Island	0.905	0.93
(100)	0.095	0.07
Leelenau Co.	0.949	0.912
(68)	0.051	0.088
Benzie Co.	0.909	0.955
(11)	0.091	0.045
Charlevoix Co.	1.00	0.97
(33)	.0	0.03
Oscoda Co.	0.96	1.00
(21)	0.04	.0
Mason Co.	0.971	0.912
(17)	0.029	0.088

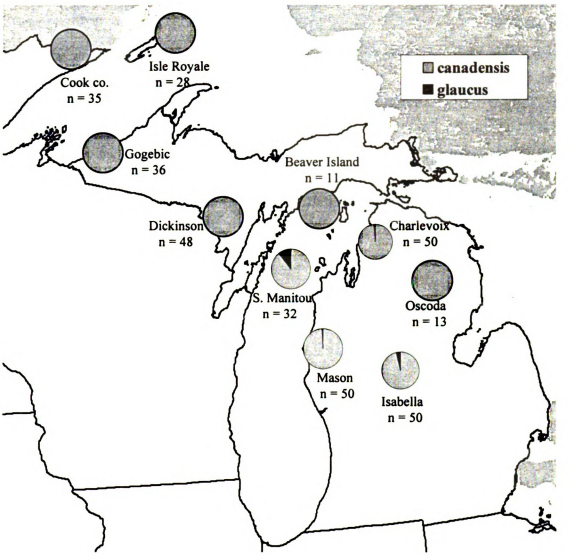


Figure 3.6. 1998 populations sampled. Pie charts indicate the percentage of *glaucus* alleles in each population for the Pgd allozyme locus.

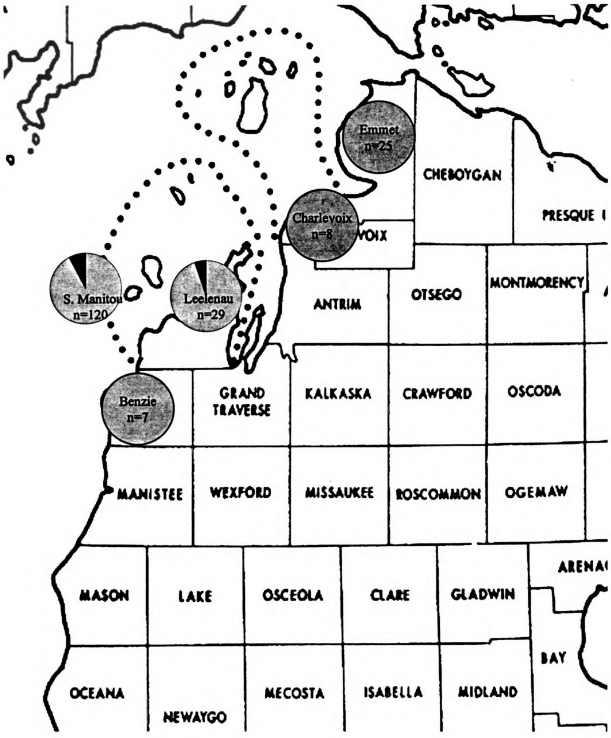


Figure 3.7. 1999 populations sampled. Pie charts indicate the percentage of *glaucus* alleles in each population for the Pgd allozyme locus.

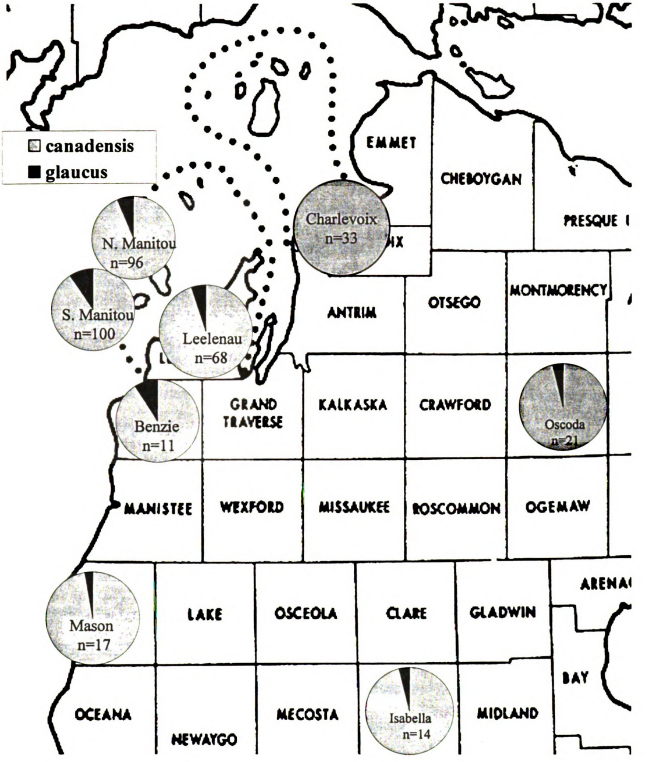


Figure 3.8. 2000 populations sampled. Pie charts indicate the percentage of *glaucus* alleles in each population for the Pgd allozyme locus.

DISCUSSION

Based upon the combined analysis of morphological and biochemical traits, I have found measurable evidence suggesting that there is a significant amount of *P. glaucus* introgression in the South Manitou Island population of *P. canadensis*. The presence of individuals heterozygous (intermediate) for even single diagnostic markers suggests hybridization. The South Manitou Island population exhibits heterozygosity (intermediacy) for multiple diagnostic characters, resulting in a "diverse array of recombinant types". This diversity of mixed ancestry can best be described as a "hybrid swarm" (Harrison 1993). This hybrid swarm however, is unusual in that it appears to be isolated at a latitude approximately 150 kilometers north of the historically described *P. glaucus* and *P. canadensis* hybrid zone (Scriber 1982; Scriber et al. 2001).

The most readily available distinguishing morphological markers between *P. glaucus* and *P. canadensis* are wing characters. Both forewing length and the relative width of the hindwing anal cell black band are consistently described as being diagnostic between the two species (Scriber 1982; Luebke et al. 1988; Hagen et al. 1991; Nielsen 1999). The South Manitou Island population of *P. canadensis* exhibits intermediacy for both of these qualitative (autosomaly inherited) characters.

Papilio glaucus has a significantly larger forewing length than does *P. canadensis* (Hagen et al. 1991). Forewing length in these *Papilio* species has been shown however to be impacted by ecological environmental conditions (Ayres and Scriber 1994) and generally correlated with latitude from Alaska to Florida (Scriber 1994; Chapter 2, Figure 2.4). Statistical analysis shows that in 1998 the forewing lengths of *P. canadensis* from

South Manitou Island were significantly smaller than a known population of *P. glaucus* from Ohio but were not significantly larger than a population of *P. canadensis* from Oscoda county, Michigan (Figure 3.2). This lack of difference of forewing lengths, between the South Manitou population and the Oscoda *canadensis* population does not support the hypothesis of *glaucus* introgression, but should be further scrutinized. However, the South Manitou Island population displayed the largest mean wing length of any *canadensis* population surveyed in 1998 (Figure 2.4) except Mason county, which lies at the northern most border of the known hybrid zone. A combination of environmental selection pressures, latitude, and *glaucus* introgression could be influencing this trait.

More indicative of *glaucus* introgression is the intermediacy of the hind wing anal cell black bandwidth displayed in the South Manitou Island population. This black bandwidth is the more consistent trait used to distinguish between *P. glaucus* and *P. canadensis* in the field. It too may be influenced by environmental conditions and latitude (Figure 2.6) but has been shown to have a significant genetic component that will be intermediately expressed in lab reared interspecific hybrid crosses. Lab reared primary hybrids from many families have a mean black bandwidths that fall between 42% and 57% (Scriber 1982). The South Manitou Island population black bandwidth was significantly different from, and intermediate between both the Ohio *glaucus* population, and the Oscoda county *canadensis* population located at the same latitude (Figure 3.3). The mean black bandwidth was 55.2%. This intermediacy between the two species for this morphologic trait is a documented method of identifying hybridization and therefore would be indicative of *glaucus* introgression in the South Manitou Island

population. The selective advantage of such black band (melanic) widening with latitude is not clear, although a role in thermoregulation can't be ruled out (Watt 1968, Kingsolver 1985, 1987, 1995).

Reciprocal host plant oviposition preferences and larval detoxification abilities have long been described for *P. glaucus* and *P. canadensis*, even before they were given distinct species status (Hagen et al. 1991). Given a choice of Tulip tree, Quaking aspen or Black cherry, *P. glaucus* females prefer to oviposit on Tulip tree, whereas *P. canadensis* prefers to oviposit on Quaking Aspen. This genetically determined choice preference appears to be a sex-linked trait contained on the X chromosome (Scriber et al. 1991; Scriber 1994). Larvae of *P. glaucus* develop poorly on Quaking aspen, a good host for *P. canadensis,* due to an inability to detoxify the phenolic glycosides that are present (Scriber et al. 1989). Conversely, Tulip tree is toxic to *P. canadensis* larvae, preventing growth, whereas it is successfully utilized by *P. glaucus* (Hagen et al. 1991). The particular toxin for *P. canadensis* in Tulip tree is not known at this time. Hybrid larvae can use both species (Scriber et al. 1995), and backcrosses have intermediate detoxification and growth rate abilities (Scriber et. 1989; Scriber et al. 1999).

During the years assayed (1998-2000), the South Manitou Island population of *P. canadensis* exhibited strong oviposition preference for Tulip tree and larval ability to detoxify and utilize this host plant (Table 3.3, Figures 3.4 and 3.5). In 1998, 80% of the females that exhibited a strong oviposition preference chose Tulip tree. 56% of all eggs laid in oviposition preference tests were laid on Tulip tree. In 1999 the results were not as striking but are still highly unusual for a *canadensis* population, with 40% of the

females exhibiting a strong oviposition preference choosing Tulip tree. Of the total eggs laid from that population 40% were placed on Quaking aspen followed closely behind by 36% being placed on Tulip tree. Again in 2000 a strong Tulip tree preference is shown. 87.5% of all females exhibiting a strong preference, and 41% of the total eggs were placed on Tulip tree. Most *P. canadensis* have much lower preference for Tulip tree in the 3-choice arena and no *P. canadensis* larvae from Canada, Wisconsin or Michigan survived on Tulip tree in previous studies (Scriber et al. 1991).

These population analyses indicate that a large portion of the total eggs oviposited by females from the South Manitou island population are placed on Tulip tree. However, upon closer analysis of the oviposition preferences of each individual female assayed, a clear dichotomy becomes apparent. The vast majority of individual females that oviposited more than 10 eggs showed a clear preference for either Tulip tree or Quaking aspen (see Appendix Table 2.1). This strong dichotomous preference could be explained through the population having mixed ancestry of *P. canadensis* and *P. glaucus.*

The results of the larval survival assay for South Manitou Island are also suggestive of *glaucus* introgression. In 1999, a striking 52% of the total larvae assayed survived on Tulip tree. In 2000, this number was only 18%. Though greatly reduced, this is still of ecological significance as the expected survival of *P. canadensis* larvae on Tulip tree is 0.0%. It should be noted that population larval survival would not necessarily directly follow the trend in oviposition preference. Oviposition preferences for Quaking aspen or Tulip tree is genetically determined basically by a single locus on the X-chromosome, while larval detoxification abilities are more complex and polygenic in derivation (Scriber 1994).

Like with the oviposition data, attention should be given to the survival rates of individual families (see Appendix Table 2.2). In 1999, out of 10 families assayed for larval survival on Tulip tree, there were only two families that had 0.0% survival. In 2000, only 40% of the families assayed had 0.0% survival rates. For both of the years that larval survival was assayed on the three host plants, 1999-2000, there is a strong indication that larvae from South Manitou females are able to survive on both Quaking aspen and on Tulip tree. The ability for larvae to survive on these two host plants follows the host plant utilization abilities of lab reared *canadensis / glaucus* hybrids (Scriber 1994).

The most convincing evidence of significant levels of *glaucus* introgression into the South Manitou Island population of *P. canadensis* are the results of the allozyme electrophoresis analysis. Allozyme electrophoresis is frequently utilized to distinguish between closely related species and to monitor introgression in and around hybrid zones (Scriber et al.1992; Harrison 1993; Johnson et al.1996; Jiggins and Mallet 2000), including *P. glaucus* and *P. canadensis* (Hagen et al. 1991; Hagen & Scriber 1991). The South Manitou Island population of *P. canadensis* shows significant levels of *glaucus* introgression at two key diagnostic loci, Pgd and Hk, for every year surveyed. The level of *glaucus* introgression for these two allozymes is relatively comparable. It would be easy to anticipate that any other diagnostic allozymes should likewise exhibit comparable levels of introgression. It could be expected that this would be especially true of the Ldh diagnostic allozyme since, like Pgd, Ldh is an X-linked allozyme. Given that these two allozymes are linked on the X chromosome, it would seem as though the levels of

introgression at these two loci should be highly correlated. This however is not the case at all. After the electrophoretic analysis of all of the samples collected north of the hybrid zone, including South Manitou Island, there is no indication of any Ldh introgression at all between 1998 and 2000 (see also Hagen 1990.).

In summary, this investigation has focused on multiple diagnostic traits for the South Manitou population of *P. canadensis*. Independent analysis of each of these traits indicates *P. glaucus* introgression. There are however other diagnostic traits between *P. canadensis* and *P. glaucus*, for which introgression is not expressed (i.e. Ldh allozyme). In fact, after analysis of over 250 male specimens from South Manitou Island, no primary hybrids appeared. This suggests two things. First, the hybrid zone is a semipermeable barrier to gene flow. And second that the introgression has been present for multiple generations.

Specific evidence that the hybrid zone must act as a semipermeable barrier to gene flow is readily apparent from the differential introgression of certain diagnostic traits. Consider the differential introgression of the Pgd and Ldh allozymes. This is especially significant in that they are both X-linked and should be highly correlated. Similarly, Tulip tree host use abilities have moved extensively northward with the last few years of regional climate warming, but aspen abilities have not moved southward (Scriber 2001). These examples of differential movement of genes must be enforced by strong environmental selection. Reasons for this differential selection are still under investigation.

It is very likely that historic periods of warming along with any "lake effect" might produce a moderated environmental passageway along the Lake Michigan coast, allowing the movement of *glaucus* genes. After the return of "normal" temperatures across the state the South and North Manitou island environments are able to maintain certain alleles due to their moderated climates. The growing season of the Leelanau Peninsula and associated islands is actually comparable to that of Lansing with an average of 157 frost-free days. Grayling, which is at the same latitude, has a growing season of only 114 days. (Haswell and Alanen, 1994). The exhibited mixed ancestry is then maintained as a result of the isolation of the islands preventing genetic swamping from the surrounding mainland gene pool.

In fact, South Manitou Island, and likely North Manitou Island, represents old genetic introgression. There are several indications that this is the case. First, as previously described, there have never been any primary hybrids encountered. A primary hybrid would be a first generation offspring resulting from an intraspecific pairing between *P. canadensis* and *P. glaucus*. Secondly, there are several specimens that scored homozygous for *glaucus*-like alleles at certain allozyme loci (see appendix). Additionally, the relatively high frequency of *glaucus*-like alleles at the Pgd locus, accompanied by the complete absence of any individuals with *glaucus*-like alleles for the other X-linked diagnostic loci (Ldh), can most simply be interpreted as a chromosomal crossover event that occurred, producing offspring with *glaucus* Pgd alleles (-100) and *canadensis* Ldh alleles (80). Evidence for such a crossover event on South Manitou Island was provided several years ago through analysis of offspring resulting from a lab hand cross pairing, of a lab reared *P. glaucus* female and a single field captured *P.*

canadensis from South Manitou Island in 1991 (Scriber, 1994). At the time of the 1991 study, the significance of the find was not fully recognized. A more comprehensive population investigation was required to identify the extent of the *glaucus*-like introgression into the *P. canadensis* population of South Manitou Island.

CHAPTER 4:

SUMMARY

To more clearly determine whether or not there is genetic differentiation between the island and mainland populations of *P. canadensis* I suggest the use of additional biochemical and alternative molecular markers. This study only utilized one highly polymorphic locus (Pgd). Use of multiple allozyme loci would be far more powerful in identifying genetic differentiation. Likewise, the use of alternative molecular markers such as RAPDs (Randomly Amplified Polymorphic DNA) or AFLPs (Amplified Fragment Length Polymorphisms) might be better suited to detect genetic differences.

Several aspects of this preliminary study indicates that further research is necessary in order to truly evaluate whether or not there is island versus mainland genetic differentiation. First, the Beaver Island population exhibited complete fixity for the Pgd allozyme for a single allele (125). This is in great contrast to Charlevoix co., the most adjacent mainland population. In 1998 Charlevoix co. exhibited the greatest allelic diversity of any of the populations that were assayed. It is possible that the apparent lack of allelic diversity on Beaver Island is the result of a low sample size (n=11) however it seems unlikely that this alone would account for the great difference.

The more compelling aspect of this research that indicates a need for further investigation is the odd finding of a "hybrid swarm" on South Manitou Island. The hybrids found there are of mixed ancestry of *Papilio canadensis* and *Papilio glaucus.* This thesis merely describes the existence of this oddity. There are questions that

logically follow remain unanswered. How has this "hybrid swarm" come to exist approximately 150 kilometers north of the described *canadensis / glaucus* hybrid zone? Why has this population of mixed ancestry been maintained on the island for multiple generations?

After three years, focusing on the South Manitou population and adjacent populations of *P. canadensis*, through extensive analyses of multiple diagnostic traits I have shown that there is a significant amount of genetic introgression not only on South Manitou Island, but also on North Manitou Island and the adjacent mainland. In addition, there is substantial evidence to suggest that this genetic introgression has been present and / or occurring for at least the past 12 years.

The South Manitou Island population of *P. canadensis* can best be described as a hybrid swarm (Harrison 1993) as it is a localized area of individuals exhibiting a diverse array of mixed ancestry. How the high levels of introgression came to be present in this population was not the focus of this study, however it seems extremely possible that it has been facilitated by historic climatic changes. As a result of a lake effect moderating the western shore of the State of Michigan, accompanied by periods of regional warming, *glaucus*-like genes would have a narrow passageway north along the western shore. When "normal" cool temperatures returned to the region, these *glaucus* genes would be maintained on South Manitou Island as a result of the moderated temperatures that that portion of the state consistently experiences. The *glaucus* genes in the mainland population of *P. canadensis* would be eliminated through a swamping effect, while the island population retained the mixed ancestry due to isolation.

As a result of the shift in focus of this research so early on, the original question, whether or not there is significant genetic differentiation between the island populations and adjacent mainland populations, was not fully attended to.

APPENDICES

APPENDIX 1:

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2001-09

Title of thesis or dissertation (or other research projects):

ISLAND POPULATIONS AND TRAIT COMPARISONS OF TIGER SWALLOWTAIL BUTTERFLIES, *P. CANADENSIS*, IN THE GREAT LAKES REGION

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Gabriel J. Ording

Date 10/4/01

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1:

VOUCHER SPECIMEN DATA

Appendix 1.1

Voucher Specimen Data

Page 1 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio canadensis	MICHIGAN North Manitou Island Leelenau co. June 8, 2000 G. Ording						5		MSU
	MICHIGAN Benzie co. June 3, 2000 G. Ording						2		MSU
	MICHIGAN Dickinson co. May 26, 1998 A. Stump						2		MSU
	MICHIGAN South Mantiou Island Leelenau co. June 18, 1998 G. Ording						5		MSU
	MINNESOTA Cook co. May 29, 1998 A. Stump						2		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Gabriel J. Ording

Date October 5, 2001

Voucher No 2001-09

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 2 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio canadensis	MICHIGAN Gogebic co. June 22, 1998 G. Ording MICHIGAN Isle Royale National Park Keewenaw co. May 28, 1998 G. Ording MICHIGAN Emmett co. June 8, 1999 G. Ording MICHIGAN Beaver Island June 14, 1998 G. Ording MICHIGAN Isabella co. May 18, 1998 J.M. Scriber						2 2 2 2 2		MSU MSU MSU MSU MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Gabriel J. Ording

Date October 5, 2001

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Appendix 1.1

Voucher Specimen Data

Page 3 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio canadensis	MICHIGAN Mason co. May 18, 1998 P. Giroux / N. Siegert						2		MSU
	MICHIGAN Oscoda co. May 17, 1998 G. Ording						2		MSU
	MICHIGAN Charlevoix co. May 17, 1998 J.M. Scriber						2		MSU
	MICHIGAN Leelenau co. May 27, 1999 G. Ording						5		MSU
Papilio glaucus	OHIO Lawrence co. May 15, 1998 J.M. Scriber						3		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Gabriel J. Ording

Date October 5, 2001

Voucher No 2001-09

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

APPENDIX 2:

POPULATION WING MEASUREMENTS AND ALLOZYME DATA

APPENDIX

Table 1. Specimen information by location and year. Each specimen allozyme data presented represents two alleles. If only one allele is indicated, the individual is homozygous for that allele. If two alleles are indicated the individual is heterozygous for those two alleles. 1998 Pgd allozyme data for Cook, taken from Stump 2000.

1998 Cooke County Minnesota

ID Male #	Forewing Length	Wings Hind Wing Black Band	Allozymes PGD
1	41	68.5	125
2	43	79	125
3	44	78.5	125
4	45	83.5	125
5	44	74	125/80
6	40	65	150/125
7	46	75.5	125
8	44	70	125/80
9	42.5	68.5	125
10	44	72	125/80
11	43	69	125
12	43	80	125
13	43.5	59.5	125
14	44.5	76.5	125
15	44	72.5	125
16	48.5	66	125
17	43.5	66	125
18	42.5	63.5	125
19	41.5	65.5	125/80
20	46	78.5	125
21	44.5	67	125
22	40	56	125
23	46	69	125
24	42.5	66	125
25	41	77.5	125
26	42.5	71	125
27	42	74	125
28	45	80	125
29	42	62.5	150/125
30	42.5	78	125

31	45	75.5	125
32	42.5	73.5	125
33	44	72	125/80
34	45	76	125
35	43	70.5	125/80

1998 Isle Royale

		Wings		Allozymes	
ID	**Forewing**	**Hind wing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Black Band**			
1	46.5	69	125	80	110
2	43	73.5	125	80	110
3	42	66.5	125	80	110
4	45	62.5	125	80	110
5	44	56	125	80	110
6	46.5	77.5	125/80	80	110
7	41.5	70	125	80	110
8	46	75.5	125	80	110
9	44	71	125	80	110
10	46.5	72.5	125	80	110
11	45	73	125	80	100
12	43	70.5	125	80	110
13	44.5	69	125	80	110
14	40	75	125	80	110
15	44	66	125/80	80	*
16	40	69.5	125	80	110
17	40	71.5	125/80	80	110
18	47	74.5	125	80	110
19	42.5	73	125	80	110
20	46	68.5	125/150	80	110
21	43	67.5	125	80	110
22	43.5	69	125	80	110
23	45	62.5	125	40	110
24	46	73.5	125/80	80	110
25	44	61.5	125	80	110
26	41	75	125	80	110
27	43	68	125	80	110
29	43	65	125/80	80	110
Female					
14109	42	68			

1998 Gogebic County - Data

ID Male #	Forewing Length	Wings Hindwing Black Band	Allozymes PGD
1	45	73.5	125
2	42	71.5	125/80
3	41	53	125
4	44.5	70.5	125
5		65	125/80
6	45.5	79.5	125
7	45	68.5	125
8	44.5	58	125
9	44.5	72	125/80
10	46	78	125
11	45	58	125
12	43	55	125
13	45.5	63.5	125
14	45.5	79	125
15	41	69	125
16	43.5	70.5	125
17	44	62.5	125
18	44.5	78.5	125
19	46	56	125/80
20	45	68	125/80
21	45.5	72.5	125
22	44	63	125
23	43	64.5	125
24	42	68	125
25	44	79	125
26	45	72	125/80
27	45	49	125/80
28	47.5	69.5	125
29		64.5	125/80
30	45	70	125/80
31	42	72	125
32	45	69	125
33	45	68	125
34	45	63.5	125
35	40.5	62.5	125/80
36	43	77	125

Female #		
14265	48	74
14266	40	73
14267	46	70.5
14268	44.5	83

1998 Dickinson County - Data

ID Male #	Forewing Length	Wings Hindwing Black Band	Allozyme PGD
1A	47	58	
2A	45	66.5	
3A	45	68.5	
4A	44	66	
5A	46.5	71.5	
6A	40.5	68	
7A	45	75.5	
8A	42	70.5	
9A	46.5	67	
10A	45	64.5	
11A	44.5	78	
12A	45	68.5	
13A	44.5	60	
14A	43.5	62.5	
15A	47	74	
1	46	63.5	150/125
2	43	78.5	125/80
3	41	69	125
4	43	69.5	125/80
5	44	63	150/125
6	43.5	67	125
7	44	59.5	125
8	47.5	70.5	125
9	44	71	125
10	45	65	150/125
11	45	69.5	125
12	43.5	69.5	125
13	45	65	125/80
14	45	65	125
15	43	55.5	125

16	42	57	150/125
17	45	68.5	125
18	44.5	73.5	125/80
19	41.5	62.5	125
20	43	67.5	125
21	46	64	125
22	41.5	66	125/90
23	45.5	59	125
24	48.5	55	125
25	45	71	125
26	46.5	69.5	125
27	42	74.5	125/80
28	43.5	66	125
29	43.5	69.5	125
30	46.5	77.5	125
31	50	63.5	125
32	44	82.5	125
33	46	56.5	125
34	47	64.5	125
35	48	62	125
36	45	61.5	125
37	44.5	72	125
38	48	72	125
39	47	64.5	125/80
40	40	68.5	125/80
41	42	72.5	125
42	46	63.5	125
43	46.5	70	125/80
44	45	57	150
45	43.5	64	125/80
46	43	71	125
47	46	68	150/80
48	44	68.5	125

1998 Beaver Island - Data

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Length**			
1	47	62.5	125	80	110
2	45	60	125	80	110
3	44	60.5	125	80	110
4	45	72.5	125	80	110

5	46.5	54	125	80	110
6	49	50	125	80	110
7	45	62.5	125	80	110
8	46	49	125	80	110
9	49	65.5	125	80	110
10	47	64.5	125	80	110
11	46.5	67.5	125	80	110
Female #					
14203	47	77	125	*	*
14204	48.5	71.5	80	80	110
14205	51		125	80	110

1998 Charlevoix County - Data

		Wings	Allozyme
ID	**FW Length**	**HW BBW%**	**PGD**
Male #			
1	46.5	56.5	125
2	44	59.5	125
3	47	76.5	125
4	47	56.5	125
5	45	49.5	125/80
6	57	74	125
7	45	64.5	125
8	42	52	125
9	44	62	125
10	45	62.5	125
11	47	59	125
12	40.5	63.5	125
13	44.5	56.5	125
14	45	62.5	125
15	42.5	73	125
16	46	60.5	125
17	46	54.5	125
18	44.5	62	125
19	48.5	60.5	125
20	46	60.5	150/125
21	47	68	125
22	46	66	125
23	50	68.5	125
24	42	63.5	125
25	46.5	57.5	150/125
26	47.5	68.5	125
27	45.5	55	125

28	46	79	125
29	44	68	125
30	45.5	57	125/80
31	44	75	125
32	43	74.5	125
33	49	53.5	125
34	49	58.5	125/80
35	50	61	125
36	45	68	125/80
37	45	70	125
38	44	68	125
39	45	43	125
40	49	53.5	125
41	44.5	59.5	150/125
42	49.5	66	125/80
43	45	61.5	125
44	46	59	125/50
45	49	54.5	125
46	44.5	63	125
47	46	65	125
48	43.5	67	125
49	49	59	125
50	47.5	65	125

Female #		
14039	53	66.5
14042	49.5	71
14037	45	62
14041	45	72.5
14036	51.5	68.5
14035	50.5	64
14040	48.5	54.5
14033	44.5	59
14034	51	71.5
14038	44	65.5
14013	48.5	55.5
14015	49	58
14014	42	75
14009	49.5	70.5
14007	47.5	76
14002	50.5	69.5
14008	48.5	61

1998 South Manitou Island

ID Male #	Wings Forewing Length	Wings Hindwing Length	Allozymes PGD	Allozymes LDH	Allozymes HK
1	49.5	61.5	125	80	110
2	49.5	55	125	80	110
3	38.5	52.5	125	80	110
4	47	54	125	80	110
5	39	53	125/100	80	110
6	46.5	60.5	125	80	110/100
7	44.5	57	125	40	110
8	49	53	100	80	110
9	49	50	125/80	* 80/40	110
10	48.5	61	125/100	80	110
11	50	73	125	80	110
12	47	38.5	125	80	110/100
13	48.5	60.5	125	80	110
14	46.5	62.5	125	80	110
15	49.5	54	125	80	110
16	49.5	49	125	80	110
17	45	52	125	80	110
18	44	43	125	80	110
19	46.5	38	125	80	110/100
20	45	62.5	125/100	80	110
21	45.5	53.5	125	80	110
22	48	52	125	80	110
23	48	60	125	80	110
24	48.5	57	125	*80	110
25	46.5	57	125	80	110
26	46.5	53	125	80	110
27	48	56.5	125	80	110
28	45	53.5	100	80	110
29	47.5	62	125	80	110
30	49	64	125	80	110
31	42.5	60.5	125	80	110
32	46	47	125	80	110

1998 South Manitou Island - Female Data

ID	Wings Forewing Length	Wings Hindwing Black Band	Allozymes PGD	Allozymes LDH	Allozymes HK
14219	51.5	59.5	125	80	*
14220	51	57.5	150	80	110
14221	55.6	55.5	125	80	*
14222	44	61.5	**	40	*
14223	51	47	**	80	*
14224	50.5	64.5	80	80	110
14225	51	57.5	100	80	*
14226	53	59	**125	80	*
14227	50.5	59.5	**125	80	110
14228	50	62.5	125	80	110
14229	55	66	*	*80	*
14230	51.5	64	125	80	110
14231	47	60	**80	80	**
14232	53.5	60.5	**	80	**
14233	51	60.5	**	80	**
14234	50	59.5	125	80	110
14235	53	55	**	80	**
14236	49.5	58.5	125	80	110
14237	51.5	69	125	40	110
14238	53	53	125	80	110
14239	51.5	55.5	100	80	110
14240	54	67.5	125	80	110
14241	50	64	80	80	110
14242	48	52.5	125	80	110

1998 Mason County - Data

ID Male #	Wings Forewing Length	Wings Hindwing Black Band	Allozymes PGD
1	47	68	125
2	48	40.5	125/100
3	48	52	125
4	48	46	125
5	47.5	69	125
6	50	64	150/125
7	51	57	150/137

8	51	66	125
9	50	53	125
10	46.5	60.5	125/80
11	44.5	63.5	125
12	49	66.5	125
13	51	55	125
14	46.5	64	125
15	45	46	125
16	43	62	125
17	48	61	125
18	49	49	150/125
19	50	42	125/80
20	49	59.5	125
21	45	53.5	125
22	48	55	125
23	50	65	125/80
24	46	58	125
25	47.5	51.5	125/80
26	46.5	59	125
27	48.5	42	125
28	46.5	57	125
29	52	61.5	125
30	47	61	125
31	46.5	57	125
32	47.5	71.5	125
33	46.5	53.5	125
34	47	60	125
35	51	55	125
36	47	59	125
37	45	70.5	125
38	49.5	52	125
39	49		125
40	50.5	63	150/80
41	48	59	125/80
42	47.5	62.5	125
43	43	65.5	125
44	47.5	60	125
45	49	66	125
46	48	63.5	125
47	54	51	125
48	42.5	65	125
49	51	73	125/80
50	48.5	51	125

Female #		
14065	48	71.5
14062	49	58
14067	49	66
14068	55	75
14060	51	73.5
14064	48	56
14070	54	52
# 7	54.5	60
# 8	49	67
14061	49	59
14071	52	61
14072	51	59
14069	49	57
14073	52	62
14074	50	64.5

1998 Oscoda County - Male Data

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Black Band**			
1	49	65	125	80	110
2	51	73.5	125	80	110
3	41	70.5	125	80	110
4	47.5	58.5	125	80	110
5	48	65.5	150/125	80	110
6	45.5	54.5	125	80	110
7	48.5	54.5	125	40	110
8	40	66	125	80	110
9	44	71	125	80	110
10	49	58.5	*150/125	40	110
11	46.5	64.5	125	80	110
12	45	63.5	125	80	110
13	43	58.5	125	80	110

1998 Isabella County - Data

ID Male #	Forewing Length	Wings Hindwing Black Band	Allozymes PGD
1	48.5	46.5	125
2	49	60	125
3	47.5	62	125
4	48.5	65.5	125
5	50	63.5	125
6	48	56	125/80
7	46	68	125
8	47	70	125
9	46.5	67.5	125
10	47.5	57	125
11	43	59	125
12	46	59.5	125
13	48	59	125/100
14	48	41	125
15	47	75	125/80
16	52	54	125
17	48	33	125
18	47.5	63	125
19	48	50	125
20	45	59	125
21	50	60	125
22	43	63.5	125
23	45	74.5	125
24	45	64.5	125
25	45	60.5	125
26	45	65.5	125
27	49	61.5	125
28	51	64.5	125
29	46	70	125
30	48	67	125
31	46	61	125/80
32	47.5	50	125/80
33	47	68	125
34	47.5	71	125
35	48.5	61.5	125
36	49	70	125
37	43.5	61.5	125
38	47	69.5	125
39	45.5	57	125

40	45.5	76.5	125
41	45	55.5	125
42	48	68.5	125
43	46	71	125/100
44	40.5	65.5	125
45	42	61	125
46	48	52.5	125/80
47	49	73.5	125/80
48	52	55.5	125
49		59	125
50	51.5	46.5	125
Female #			
14000		65.5	
14020	47.5	65	
14021	46	57.5	
14022	47	65.5	
14023	47	61.5	
14024	51	63	
14025	51	61.5	
14026	49.5	49	
14027	49.5	84.5	
14028	52	62.5	
14110	46	69.5	
14111	51	63	
14112	50	66	
12–98	45	69	

1998 P. glaucus Lawrence County, Ohio

ID Male #	Forewing Length	Wings Hindwing Length
1A	54	39
2A	48	37.5
3A	43.5	31
4A	51	19
1	49	37.5
2	47	38
3	52	36
4	50	39.5
5	45	46
6	48.5	46

7	50	40
8	49	39
9	52.5	10.5
10	46	49
11	49	25
12	48.5	52
13	50	42
14	49	25.5
15	49	44
16	51	25.5
17	50	41
22	46.5	33.5

1999 Emmet County

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Length**			
1	45	65.5	125	80	110
2	47.5	63.5	125	80	100
3	45	64.5	150/125	80	110/100
4	49	59	125	80	110
5	46	66	125	80	110
6	49	65	125	80	110
7	44.5	61.5	125	80	110
8	43.5	67	125	80	110/100
9	43.5	49	125	80	110
10	46	61	125	40	110
11	48	72.5	125	80	110
12	47.5	63	125	80	110
13		58.5	125	80	110
14	45.5	56.5	125	80	110
15	46.5	63	125	80	110
16	44.5	75	125	80	110
17	46.5	54	150/125	80	110
18	48	61.5	125	80	110
19	47	70	125	80	110
20	48	72	125	80	110
21	48.5	67.5	125/80	80	110
22	46	63	125	80	110
23	46	64.5	125	80	110
24	48	66.5	125	40	110

Female ID		
15119	51	75.5
15120	50	65
15120	46	60
15153	50	78.5
15154	45	59
15155	47	75
15156	48	60.5
15157	49	62.5

1999 Charlevoix County - Data

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Black Band**			
1	48	50.5	125	80	110
2	48	74	125	80	110
3	44	58	125	40	110
4	46	67	125	80	110
5	43.5	70	125	80	100
6	48	64	125	80	110
7	45	59.5	125	80	110
8	47	73.5	125	80	110
9	47.5	61.5	125/80	80	*
10	41.5	59	125	80	*
11	44.5	72.5	125	40	*
12	45	59	125	40	*
13	47.5	54	125	40	*
14	48	56	125/80	80	*
15	43	75	125	80	*
16	44	71	125	80	*
17	45	62.5	125	80	*

Female #

15149	45	70.5	125	*	*
15151	49	55.5			

1999 South Manitou Island - Male Data

ID	Wings		Allozymes		
	Forewing Length	Hindwing Black Band	PGD	LDH	HK
1	48	51	125	80	110
2	46.5	65	125	80	110
3	51	55	125	80	110/100
4	46	55	125/80	80	110
5	46	67	125	80	110
6	44.5	55	80	80	110
7	40.5	60	150/100	80	110
8	45	56	125/80	80	110
9	52.5	36	125	80	110
10	45	53	125	80	110
11	46	64	125	80	110
12	42.5	52	125	80	110
13	46	62	125	80	110
14	50	60	125	80	110
15	47	51	125	80	110
16	46	57	125	80	110
17		57	125	40	110
18	48	66	125/100	80	110
19	49.5	52	125/80	80	110
20	49	66	125/100	80	110
21	46	56	125	80	110
22	44.5	56	125	80	110
23	49.5	52	125	80	110
24	51.5	50	125	80	110
25	52.5	60	125	80	110
26	47.5	64	125	80	110
27	49.5	57	125	40	110
28	48	58	125	80	110
29	48	58	100/50	80	110/100
30	47	72	125/100	80	110
31	48.5	66	125	40	110
32	50	60	125	80	110
33	47	55	125	80	110
34	50	53	100/50	80	110
35	54.5	54	125	80	110
36	43.5	54	125	80	110
37	50	65	125	80	110
38	51	52	125/80	*100/80	110

39	48.5	57	125/100	80	110
40	47.5	59	125	80	110/100
41	47.5	58	125/80	80	110
42	52	62	125	80	110
43	46	61	125	80	110
44	50	59	125/100	80	110
45	46.5	66	125/100	80	110
46	48	68	125	80	110
47	52.5	60	125/80	40	110
48	47	45	125	80	110
49	49.5	54	125/80	80	110
50	49.5	62	125	80	110
51	50.5	52	125	80	110
52	46	58	125/80	80	110/100
53	50	66	125	80	110
54	50	54	125	80	110
55	48	61	125/100	80	110
56	48	52	125/80		110/100
57	52.5	53	125	80	110
58	49	61	125	80	110/100
59	44	49	125	80	110
60	49	45	125	40	110
61	49.5	58	125/100	80	110
62	51	51	150/125	80	110/100
63	50	48	125	80	110
64	50	59	125/80	80	110
65	49.5	57	125	80	110
66	48.9	54	125	80	110
67	46	60	125	80	110
68	45	74	125	80	110
69	47	63	125	80	110
70	49	48	125	80	110
71	47.5	62	125	80	110
72	46.5	67	125	80	110
73	46.5	64	125	80	110
74	49.5	55	125	80	110
75	51	61	125/100	80	110/100
76	48.5	59	125	80	110
77	50.5	44	125	80	110
78	46.5	55	125	80	110
79	48	47	125/100	*80	110
80	48.5	62	125	80	110
81	52.5	45	125	80	110
82	50	63	150/80	80	110/100
83	52.5	50	125/80	80	110/100

84	49	56	125	80	110/100
85	47	54	125/80	80	110
86	46	49	125	80	110
87	45.5	59	125	80	110
88	46	54	125	40	110
89	50	50	125/100	80	110
90	48	47	125/100	80	110
91	49	50	125	40	110
92	45	55	125	80	110
93	51	59	125	80	110
94	47.5	56	125/100	80	110
95	50.5	43	125	40	110
96	46	58	125	80	110
97	47.5	65	125	80	110
98	49	56	125	80	110
99	47	62	125	80	110/100
100	48.5	58	125	80	110
101	49	53	125	80	110
102	49.5	52	125	80	110
103	50	46	125	80	110
104	45	52	125/100	80	110
105	49.5	50	125	80	110
106	46	63	125/80	80	110
107	51	58	125	80	110
108	51	58	125/80	80	110
109	44.5	53	125	80	110
110	48	59	125	80	110
111	54	50	125	40	110
112	47	48	125	80	110
113	46	46	125	80	110
114	49	54	125	40	110
115	51	53	125	80	110
116	47.5	49	150/125	80	110
117	48	56	125	80	110
118	50	73	125	80	110
119	47.5	39	125	40	110
120	47	59	125	80	110/100

1999 South Manitou Island Female Data

ID Female #	Forewing Length	Wings Hindwing Black Band	PGD	Allozymes LDH	HK
1F-99	49	60	125	40	110
15222	50	50	125	80	110
15223	53	59	100	80	110
15224	48	62.5	125	80	110
15225	46	68	137	80	110
15226	49.5	54	125	80	110
15227	50	57.5	125	80	110
15228	46	71	125	80	110
15229	44	60.5	125	80	110
15230	50	64.5	125	80	**110
15231	53	67	125	80	110
15232	49	52.5	80	40	110
15233	45	63.5	125	80	110
15234	51	68	**	80	**
15235	49	67	125	80	110
15236	50	62.5	80	80	110
15237	53	69	100	80	110
15238	48	47	**	**	**110
15239	49	65.5	**	80	110
15240	50	66	125	80	110
15241	52	62.5	125	80	110
15242	48	69	80	80	110
15243	48	71	125	80	110
15244	50.5	65.5	125	80	110
15245	50.5	74	125	80	110
15246	54	65.5	125	40	110
15247	50	48	125	80	110
15248	47	64.5	125	80	110
15249	48	70	125	80	110
15250	52.5	60.5	80	80	110
15251	50.5	56	125	80	110
15252	48.5	62.5	125	80	110
15253	53	70	125	80	110
15254	48	59	125	80	110
15255	48.5		125	80	*
15256	51	51.5	100	80	110
15257	54	49	125	80	110
15258	49	68	125	80	110

15259	51	41.5	125	80	110
15260	49	66	80	80	110/100
15261	50	68	125	80	110
15262	54	62.5	100	80	110
15263	49.5	59.5	125	80	110
15264	46	60	125	80	110
15265	52	47.5	125	80	110
15266	51	78	125	80	110
15267	49	71.5	80	80	110
15268	52	63	125	80	110
15269	49.5	66	125	80	110
15270	51		125	80	110
15271	52	69.5	125	80	110
15272	49	59	*	40	*
15273	49	65	*	*	110

1999 Leelanau County Data

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Black Band**			
1	43	56	125	80	110
2	44	69	125/80	80	110
3	47	61.5	125	80	110
4	50	53	125	80	110
5	48	63.5	125	80	110
6	47.5	39	125/80	80	110
7	49	77	125	80	110
8	45	68.5	125/100	*80/40	110
9	46.5	63	125	80	110
10	48.5	67	125/80	80	110
11	39.5	54	125/80	80	110
12	47	57	125	80	110
13	45.5	47.5	125/80	80	110
14	43	56	125	80	110
15	48.5	60	125/80	80	110
16	49.5	58.5	125	80	110
17	49	51.5	125	80	110
18	48.5	59.5	125/80	80	110/100
19	42	69.5	125	80	110
20	48	71.5	125/100	80	110
21	49.5	58	125	80	110/100
22	46.5	67	125/80	40	110
23	46.5	64.5	125	80	110

24	47	48	125	80	110
25	46	57	125	80	110
26	42.5	60	125	80	110
27	46	64.5	125	80	110
28	44.5	62.5	125	80	110
29	46		125/100	80	110

Female #

15105	51	66
15106	45	67.5
15107	52	73

1999 Benzie County Data

		Wings		Allozymes	
ID	**Forewing**	**Hindwing**	**PGD**	**LDH**	**HK**
Male #	**Length**	**Black Band**			
1		64.5	125/80	80	110
2	46	57	125	80	110/100
3	44.5	61	125	40	110
4	46.5	55.5	125/80	80	110
5	45.5	69	125	80	110
6	47	75	125	80	110
7	46.5	59.5	125	40	110

2000 Benzie County

		Allozymes	
ID	**Pgd**	**Hk**	**Ldh**
Male #			
1	125	110	
2	125	110	
3	125	110	
4	150/125	110	
5	125/80	110	
6	100/80	110	
7	125	110	
8	125	110	
9	125	110	
10	125/100	110/100	80
11	125	110	

2000 Leelenau County

ID	Allozymes Pgd	Hk	Ldh
Male #			
1	137	110/100	
2	125	110	
3	125	110	
4	125/100	110	80
5	125	110/100	
6	125	110	
7	125	110	
8	125	110	
9	125	110/100	
10	125/80	110/100	
11	125/100	110	80
12	125	110	
13	125/100	110	80
14	150/125	110	
15	125	110	
16	125	110	
17	125/100	110	80
18	80	110	
19	125	110/100	
20	125	110	
21	125/80	110	
22	125	110	
23	80	110	
24	125	110	
25	125	110	
26	125	110	
27	150/125	110	
28	125/80	110	
29	125	110/100	
30	125	110	
31	150/125	110	
32	125	110	
33	125	110	
34	125	110	
35	125	110	
36	125	110	
37	125	110/100	
38	125	110	

39	125	110/100	
40	125	110	
41	125	110	
42	125	110	
43	125	110	
44	125/80	110	
45	125	110	
46	125/100	110	80
47	125	110	
48	125/100	110	80
49	125	110	
50	125	110	
51	125	110	
52	125/80	110	
53	125	110	
54	125	110	
55	125	110	
56	125/100	110	80
57	125	110	
58	125	110	
59	125	110/100	
60	125	110/100	
61	125	110/100	
62	125	110	
63	125	110/100	
64	125	110	
65	125	110	
66	125	110	
67	125/80	110	
68	150/125	110	

2000 Mason County

		Allozymes	
ID	**Pgd**	**Hk**	**Ldh**
Male #			
1	125/80	110	
2	125	110	
3	125	110	
4	125	110	
5	125	110/100	
6	125	110	
7	125	110	
8	125	110	

9	125	110	
10	125	110	
11	125	110	
12	125	110	
13	150/125	110	
14	125	110	
15	125/100	110	80
16	137	110/100	
17	125	110/100	

2000 North Manitou Island

		Allozyme	
ID	**Pgd**	**Hk**	**Ldh**
Male #			
1	150/125	110	
2	150/125	110/100	
3	125	110	
4	125	110/100	
5	125/100	110	
6	125/80	110	
7	125	110	
8	125	110	
9	125	110	
10	125	110	
11	125/100	110	
12	125	110	
13	125/80	110	
14	125	110	
15	125	110	
16	125	110	
17	125	110	
18	150/125	110	
19	150/125	110	
20	125	110	
21	150/125	110	
22	125	110	
23	125/100	110	
24	125	110	
25	125	110	
26	125	110	
27	125	110	
28	125	110	

29	125/80	110
30	125	110
31	125	110
32	125	110
33	125	110
34	125	110
35	125	110/100
36	125	110
37	125	110
38	125	110
39	125/80	110
40	125	110
41	125	110
42	125/100	110
43	125	110
44	125/80	110
45	125	110
46	125	110/100
47	125	110
48	125	110
49	125	110
50	125	110
51	125	110
52	125	110
53	125	110
54	125	110/100
55	125/80	110
56	125	110
57	125	110
58	125	110
59	125	110
60	125	110
61	125	110
62	125	110
63	125	110
64	125	110
65	125	110
66	125	110/100
67	125	110/100
68	125	110
69	125	110
70	125	110
71	125/80	110
72	125/80	110
73	125/100	110

74	125	110/100	
75	125/100	110/100	
76	125	110	
77	125/100	110	
78	125	110	
79	125/100	110	
80	125	110	
81	125	110	
82	125	110	
83	125	110	
84	125	110/100	
85	125	110	
86	125	110	
87	125	110/100	
88	125	110	
89	125	110/100	
90	125/80	110	
91	125	110	
92	125/100	110	40
93	125	110/100	
94	125/100	110	80
95	125/100	110	
96	125/100	110	

2000 South Manitou Island

	Allozymes	
ID	**Pgd**	**Hk**
Male #		
1	125	110
2	125	110/100
3	125	110
4	125/100	110
5	125	110
6	125	110
7	125/100	110
8	125	110
9	125/100	110
10	125	110
11	125/100	110
12	125	110
13	125	110

14	125	110
15	125	110
16	125	110
17	150/125	110
18	125	110
19	125	110
20	125	110
21	125/100	110
22	125	110
23	137/125	110
24	125	110
25	125	110
26	125	110
27	125/80	110/100
28	125/100	110
29	125	110/100
30	125/80	110/100
31	125	110
32	125/100	110
33	125	110
34	150/100	110/100
35	125/100	110
36	125	110
37	125/100	110
38	137/125	110
39	150/125	110/100
40	125	110
41	125	110
42	150/125	110
43	125/100	110
44	125	110
45	125/80	110/100
46	125/100	110
47	125	110/100
48	125/100	110
49	125	110/100
50	125	110
51	125/80	110
52	125	110
53	125	110
54	150/125	110
55	125/80	110
56	125/100	110
57	125	110/100
58	125	110

59	125	110
60	125	110
61	125	110
62	125	110
63	125	110
64	125	110
65	125	110
66	125	110
67	125	110
68	125/100	110
69	125	110
70	125/80	110
71	125/100	110
72	125/100	110
73	125	110
74	125/80	110
75	125/80	110
76	125	110
77	125	110
78	125	110
79	125	110
80	125/100	110
81	125	110
82	125	110
83	125	110
84	150/125	110
85	125	110
86	150/125	110
87	125	110
88	125/80	110/100
89	125	110/100
90	125	110
91	125	100
92	125	110
93	125/80	110
94	125	110
95	125	110
96	125	110
97	125	110
98	125/100	110
99	125	110
100	125	110

APPENDIX 2.1

OVIPOSITION PREFERENCE DATA

Appendix Table 2.1. South Manitou Island female 3-choice oviposition preference data for 1999-2000. Total numbers of eggs laid by each female are given as well as the percent of the total number of eggs placed on each of three host plants. The other category includes eggs that were placed < 2 cm from the host plant. These wer likely intended to be placed on the plant, but for the purposes of this study have been scored as *other*. Oviposition was only scored for females laying >10 eggs. Female oviposition preference was assigned only to females that layed 50% or more of her eggs on any single host plant.

1999		**Percent Eggs Laid**			
Female ID	Total Eggs (n)	Quaking aspen	Tulip tree	Black cherry	Other
1F-99	0				
15222	0				
15223	5	20	40	20	20
15224	0				
15225	0				
15226	0				
15227	**98**	17	**61**	13	8
15228	**23**	**96**	4		
15229	2	100			
15230	3		100		
15231	2		50	50	
15232	5	100			
15233	0				
15234	**13**	8	**69**		23
15235	0				
15236	0				
15237	0				
15238	1		100		
15239	**49**	2	**76**	2	20
15240	1	100			
15241	0				
15242	**22**	**82**	9		9
15243	**51**	**59**	27	4	10
15244	0				
15245	0				
15246	7	28	71		
15247	7	14	71		14
15248	0				
15249	0				
15250	**10**	**70**	10		20
15251	**54**	15	**52**	6	28
15252	2	50		50	
15253	**117**	21	40	18	21
15254	**117**	38	17	3	42

Appendix Table 2.1 continued.

15255	0				
15256	7	43	57		
15257	5	40		60	
15258	0				
15259	1	100			
15260	0				
15261	**103**	4	**81**	6	10
15262	0				
15263	2		50		50
15264	**118**	**77**	6	4	13
15265	**14**	**50**	14	7	21
15266	**110**	**84**	7	1	7
15267	**69**	39	39		22
15268	3	67		33	
15269	**21**	**52**		29	19
15270	0				
15271	6		17		83
15272	**62**	5	**73**	15	8
15273	**51**	**61**	14	8	18

2000

		Percent Eggs Laid			
Female ID	Total Eggs (n)	Quaking aspen	Tulip tree	Black cherry	Other
16136	**56**	0	**50**	32	18
16137	**81**	20	**63**	6	23
16173	3	0	67	0	33
16174	1	0	0	100	0
16175	**21**	19	**71**	5	5
16176	0	0	0	0	0
16177	**90**	7	**59**	11	23
16178	**79**	19	**54**	14	13
16179	0	0	0	0	0
16180	0	0	0	0	0
16181	**14**	7	7	36	50
16183	**61**	11	10	61	18
16184	0	0	0	0	0
16185	**36**	11	17	44	28
16186	0	0	0	0	0
16187	1	0	100	0	0
16188	0	0	0	0	0
16189	9	0	0	44	56
16190	**41**	5	41	20	34
16191	**131**	2	7	45	47
16192	**100**	19	39	12	30

Appendix Table 2.1 continued.

16193	0	0	0	0	0
16194	3	0	67	0	33
16195	0	0	0	0	0
16196	0	0	0	0	0
16197	**34**	3	**65**	18	15
16198	**64**	28	41	25	6
16199	**53**	28	25	26	21
16200	0	0	0	0	0
16201	0	0	0	0	0
16202	**190**	14	**59**	13	14
16203	0	0	0	0	0
16204	0	0	0	0	0
16205	0	0	0	0	0
16206	0	0	0	0	0
16207	0	0	0	0	0
16208	0	0	0	0	0
16209	0	0	0	0	0
16210	0	0	0	0	0
16211	0	0	0	0	0
16212	0	0	0	0	0
16213	0	0	0	0	0
16214	0	0	0	0	0
16215	**11**	9	0	**73**	18
16216	0	0	0	0	0
16217	0	0	0	0	0
16218	0	0	0	0	0
16219	**58**	3	34	41	21
16220	9	33	22	44	0
16221	0	0	0	0	0

APPENDIX 2.2

LARVAL HOST PLANT SURVIVAL DATA

Appendix Table 2.2. 1999 and 2000 South Manitou Island larval host plant survival larval host plant survival data. The number of larvae from each mother placed on each host plant is provided next to the mother ID number, followed by the percenta percentage of those larvae that survived through the first instar. Survival values > 0% for Tulip tree are in bold print as they are significant values. *P. canadensis is* not normally able to detoxify Tulip tree.

1999		%Larval Survival on each Host Plant				
ID	**n**	**QA**	**n**	**TT**	**n**	**BC**
Female #						
1F-99	0		0		0	
15222	0		0		0	
15223	0		1	**100**	0	
15224	20	75	22	**50**	29	76
15225	0		0		0	
15226	0		0		0	
15227	0		0		0	
15228	1	100	2	**50**	1	100
15229	0		0		0	
15230	0		1	0	2	100
15231	0		0		0	
15232	0		0		0	
15233	0		0		0	
15234	2	50	3	**33**	2	100
15235	0		0		0	
15236	0		0		0	
15237	0		0		0	
15238	0		0		0	
15239	1	100	2	**100**	1	100
15240	0		0		0	
15241	0		0		0	
15242	0		0		2	100
15243	1	0	2	**50**	3	67
15244	0		0		0	
15245	0		0		0	
15246	0		0		0	
15247	0		0		0	
15248	0		0		0	
15249	0		0		0	
15250	0		0		0	
15251	15	80	16	**56**	15	87
15252	0		1	0	1	100
15253	0		0		0	
15254	40	67.5	50	**48.77778**	56	92.22222
15255	0		0		0	
15256	0		0		0	
15257	0		0		0	
15258	0		0		0	
15259	0		0		0	

Appendix Table 2.2 continued.

15260	0		0		0	
15261	0		0		0	
15262	0		0		0	
15263	0		0		0	
15264	0		0		0	
15265	0		0		0	
15266	0		0		0	
15267	0		0		0	
15268	0		0		0	
15269	0		0		0	
15270	0		0		0	
15271	0		0		0	
15272	0		0		0	
15273	0		0		0	

2000		%Larval Survival on each Host Plant				
	n	**QA**	**n**	**TT**	**n**	**BC**
16136	9	44	9	0	11	73
16137	15	87	20	**35**	8	88
16173	0	0	0		2	100
16174	0	0	0		0	
16175	0	0	0		0	
16176	0	0	0		0	
16177	1	100	5	**20**	2	67
16178	25	68	25	**12**	24	10
16179	0	0	0		0	
16180	0	0	0		0	
16181	4	100	0		5	40
16183	16	50	15	0	18	56
16184	0	0	0		0	
16185	9	89	5	**60**	7	29
16186	0	0	0		0	
16187	0	0	0		0	
16188	0	0	0		0	
16189	0	0	1	0	6	67
16190	9	67	14	0	6	67
16191	37	76	34	**24**	31	81
16192	22	46	20	**45**	26	58
16193	0	0	0		0	
16194	0	0	3	0	0	
16195	0	0	0		0	
16196	0	0	0		0	
16197	2	50	0		2	100
16198	16	63	15	**40**	13	46
16199	13	85	16	**13**	12	67
16200	0	0	0		0	
16201	0	0	0		0	
16202	60	52	63	**4.8**	54	41

Appendix Table 2.2 continued.

16203	0	0	0		0	
16204	0	0	1	0	0	
16205	0	0	0		0	
16206	0	0	0		0	
16207	0	0	0		0	
16208	0	0	0		0	
16209	0	0	0		0	
16210	0	0	0		0	
16211	0	0	0		0	
16212	0	0	0		0	
16213	0	0	0		0	
16214	0	0	0		0	
16215	5	60	5	60	0	
16216	0	0	0		0	
16217	0	0	0		0	
16218	1	100	0		0	
16219	1	0	2	0	2	0
16220	4	100	2	50	3	100
16221	0		0		0	

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