Trait Cline Dynamics in Hybrid Zones and Hybrid Swarms

Doctoral Program Proposal
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System Background

Papilio canadensis and *Papilio glaucus* are closely related butterflies, but are distinct species (Hagen et al. 1991). Their combined ranges, *P. glaucus* in the south and *P. canadensis* to the north, extend from the tip of Florida to Alaska. Several diagnostic characteristics (morphologic, ecological, physiological, and biochemical) are extremely useful in distinguishing between these two species (Table 1.1; Scriber 1990). Where the ranges of these two butterflies meet, a very narrow zone of hybridization occurs. Historically, in Michigan this hybrid zone has been found between 43° and 44° latitude and had remained stable for at least two decades (Scriber 1982; Scriber et al. 1996; Figure 1.1). This narrow zone of hybridization had been coincidental with the transitional ecotone between the temperate deciduous and the boreal forest biomes. This zone of transition also coincides with the northernmost extent to which sufficient annual thermal accumulation occurs, allowing for the bivoltine physiology of *P. glaucus.*

Table 1.1. Summary of species differences discussed between *Papilio glaucus* and *P. canadensis.* (Modified from Table 1 in Scriber 1990).

Characteristic	*glaucus*	*canadensis*
(Morphological)		
Adult size (forewing length)	Large	Small
Hindwing anal cell black band	Narrow	Wide
Dark Morph Females (Y-linked)	Yes	No
(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
Diapause (X-linked)	Facultative	Obligate
Life History	Multivoltine	Univoltine
(Molecular)		
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
Dark Morph Suppressor (X-linked)	No	Yes

It has been suggested that these two species arose through genetic differentiation in allopatry during the Pleistocene ice age. With the spread of the ice sheets over vast portions of North America, a small relic population, that eventually became *P. canadensis*, was isolated in the far northwestern Berengial refuge (Scriber 1988, Scriber et al. 1991). The retreat of the ice sheets then allowed for the ranges of these two populations to again meet.

Extensive laboratory research conducted on *P. glaucus* and *P. canadensis*, using methods of hand pairing, has consistently resulted in viable hybrid offspring. As might be expected (Harrison 1993), these heterozygous hybrid individuals are variable and frequently intermediate for some of the species diagnostic characters (Scriber et al. 1995). Likewise in the wild, though rare in occurrence (<10%), some specimens field captured from the area of secondary contact appear to be "hybrid-like", exhibiting intermediacy for some of the diagnostic traits described, but not all of them (Table 1.1; Scriber 1990).

Given that these two species are able to successfully pair in the wild, producing viable offspring, what has prevented a gradual panmixis of their respective gene pools? Extensive research has been conducted to investigate what factors, both prezygotic and postzygotic, have potentially served to maintain the historically narrow zone of hybridization. It has been shown that reproductive isolation is not likely maintained through mate choice preference. In fact given a choice in field experiments, *P. canadensis* males have actually been shown to preferentially choose *P. glaucus* females (Deering and Scriber 2002). Sperm precedence studies, conducted with multiply mated females, indicate that there is no significantly higher rate of fertilization from conspecific males (Stump and Scriber 2003). As described above, offspring resulting from interspecific crosses result in viable and fertile offspring, and yet hybrid-like individuals are rare in nature. Therefore, there must be some postzygotic factor(s) responsible for the maintenance of the narrow hybrid zone and the elimination of individuals with mixed ancestry.

The most likely factor preventing genetic introgression in either direction is likely a difference in thermal environments. *P. glaucus* exhibits a multivoltine life cycle throughout its range. The northernmost populations of *P. glaucus* are bivoltine, completing two generations per year. The minimum thermal requirements to accomplish bivoltinism for *P. glaucus* are 2500-2700 F degree days (base 50° F) (Scriber 1982). Less annual thermal accumulation than this, and the second generation will not be able to complete development to pupation. In addition, field studies have been conducted that indicate that *P. glaucus* pupae are less cold tolerant than are pupae of *P. canadensis*. *Glaucus* pupae have been shown to experience increased mortality under the thermal environments naturally encountered in the *P. canadensis* home range (Kukal et al. 1991). Conversely, it has been shown that short periods of high temperatures (> 36°C), common south of the hybrid zone, can induce thermal stress, killing pupae of *P. canadensis* (Scriber et al. 2002). It has been suggested that these thermal constraints might be the central mechanism preventing southward introgression of *P. canadensis* genes and northward movement of *P. glaucus* genes.

Evidence of Extensive Introgression

Research conducted over the past few years, in and adjacent to the Great Lakes region of North America, indicates that there are increasing levels of introgression of *Papilio glaucus* genes

northward into historically "pure" populations of *P. canadensis*. Masters thesis research conducted (1998-2000) on and adjacent to the Manitou Islands in Lake Michigan, identified an isolated "hybrid swarm" over 150 km. north of the historic hybrid zone in Michigan (Ording 2001, see abstract below). Specimens collected from this hybrid swarm exhibited intermediacy for each of the species diagnostic characters. Long term monitoring of host plant use abilities across the Great Lakes region have indicated an extensive northward advancement of the Tulip tree detoxification abilities (autosomally linked) far beyond the historic hybrid zone (Scriber 2002). Likewise, allozyme electrophoresis has more recently been conducted on a series of mainland *P. canadensis* populations far north of the hybrid zone, and they too have begun exhibiting low levels of introgressed "*glaucus*-like" alleles [Pgd (x-linked) and Hk (autosomal)] (Scriber and Ording *unpublished*). Lastly, specimens collected from Oscoda co. Michigan in 2001, that were thought to be pure *P. canadensis*, were sent to a collaborating lab for use as a reference group for mtDNA analysis on *Papilio*. One of these Oscoda co. specimens was found to possess *glaucus*-like mtDNA (Andolfatto *personal communication)*. This same specimen appeared morphologically "*canadensis*-like", and when analyzed using electrophoresis, each of the species diagnostic allozymes expressed *canadensis*-like alleles.

"False Second Generation"

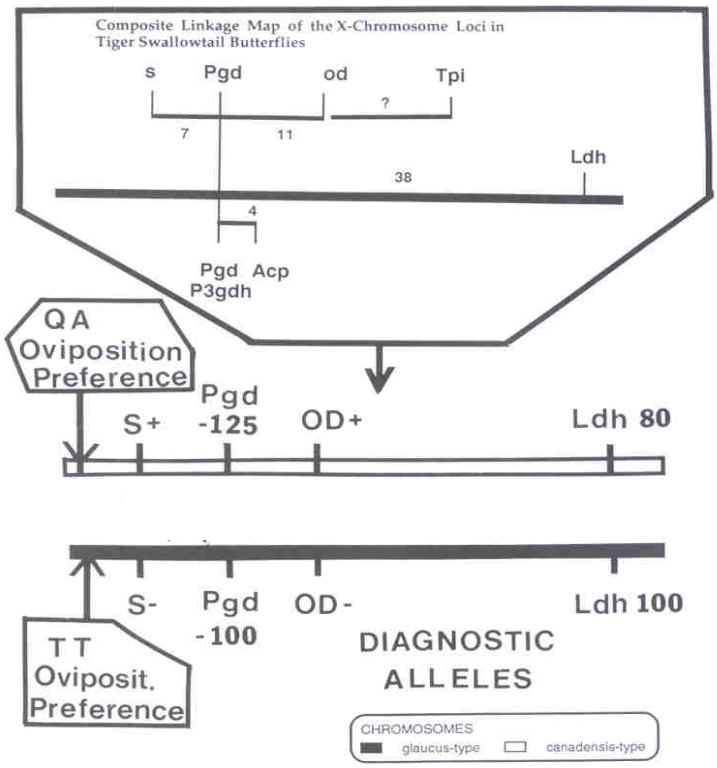
Populations of *P. canadensis* outside of the Great Lakes have also begun to show evidence of high levels of *P. glaucus* introgression. *P. canadensis* populations in central New York State and southwestern Vermont, have recently been exhibiting a highly enigmatic seasonal phenomenon. These locations have displayed what is being described as a "False Second Generation" (Scriber et al. 2003). As described earlier, *P. canadensis* has a univoltine life cycle. These New England locations however, have what appear to be two distinct flights within a single summer. The first flight occurs as would be expected (late May – June). The second of these two flights is being described as a "false second generation" due to the fact that it appears far too quickly in the season (mid July) to be a true second flight, derived from the first. This second flight is composed of individuals that appear more "*glaucus*-like" than does the first flight, being larger and possessing narrower hind wing black bands. In addition, individuals from this second flight appear "*glaucus*-like" for other species diagnostic traits (oviposition preference, tulip tree detoxification abilities, Pgd and Hk allozymes). In fact, this second flight, in stark contrast to the first flight but strikingly similar to the Manitou Island population in Michigan, might best be described as a hybrid swarm.

Asymmetrical Patterns of Gene Flow

In all of the *P. canadensis* populations that have begun to exhibit *P. glaucus* introgression, there appears to be a consistent pattern of "*glaucus*-like" traits, both present and absent. Each of these populations possesses individuals, who score either intermediate or *glaucus*-like for most of the species diagnostic traits (forewing length, hind wing black-band width, oviposition preference, tulip tree detoxification ability, Pgd and Hk allozymes). However, none of the individuals analyzed (hundreds) from these populations have possessed the *glaucus* X-linked Ldh allele (100). Nor have there been any individuals captured that have demonstrated the ability for direct development, which is dictated by the X-linked od- gene allowing for facultative diapause.

The latter of these two traits not being present is not so surprising. The offspring of any individual possessing the od- gene, that underwent direct development, would stand no chance of completing development to pupation. As earlier stated, completion of two full generations within a single year requires a minimum of 2500 degree days. On average, there are simply insufficient annual thermal units available north of the hybrid zone. This thermal constraint would likely act to strongly select against the od- gene.

As described in Table 1.1, studies of diagnostic traits in these two *Papilio* species have determined that five species-specific genetic differences exist on the X-chromosome (Scriber 1994, Hagen and Scriber 1995). The sequence in which these loci exist along the length of the X-chromosome, as well as an estimated relative map distance between each locus, is depicted in Figure 1.1 below.



Individual specimens have been field collected from these introgressed populations that have exhibited traits that suggest that they possess recombinant genotypes of X-linked diagnostic traits. There are two potential methods by which an individual could express an apparently non-concordant genotype. One method by which apparently recombined genotypes could arise is through segments of the X-chromosome being translocated to an autosome, and in this way passed on to offspring. Sex chromosome segment translocation has been described in the Mediterranean flour moth (Marec et al. 2001). A more commonly described method by which recombinant types could arise is through chromosomal crossovers during meiosis.

Direct Evidence of Chromosomal Cross Overs

The most abundant source of evidence suggesting that chromosomal cross overs occur in these *Papilionidae* are the scores of individuals field captured from these introgressed *P. canadensis* populations, that when analyzed through allozyme electrophoresis, exhibit the "*glaucus*-like" Pgd allele (100) but one of the "*canadensis*-like" Ldh alleles (40 or 80). In addition, several lab reared crosses have produced offspring expressing X-linked trait combinations that can most readily be explained through chromosomal crossovers (Scriber 1994).

Most recently, a lab-reared backcross [#18006 - *P.g. x (P.g. x P.c.)*] provided a large number of offspring that exhibited mixed X-linked traits and allowed for the mapping of the allelic combinations, including crossovers that were present in each of the offspring. This was possible to a large degree by chance, as a result of somewhat rare alleles (Pgd 50 and Ldh 40) being present in the parental generation (see 18006 back cross data in appendix). Analysis of the chromosome maps derived for each individual from this brood provides insight as to where along the length of the X-chromosome crossovers are most likely to occur, and is also suggestive as to what combinations are possible.

Why is there zero introgression of "*glaucus*-like" Ldh (100)?

Given the apparent frequency with which chromosomal crossovers can occur in these hybridizing butterflies, why is it then that the "*glaucus*-like" Ldh 100 allele is never expressed in individuals sampled from the introgressed populations of *P. canadensis*? One possible explanation is that there is direct selection on the Ldh gene. Usually, allozymes are considered to be neutral genetic markers. However, it has been shown in other systems (fish) that lactate dehydrogenase (Ldh) is a metabolic enzyme that can exist in various forms. These various forms have been shown to be differentially selected upon in differing thermal environments. It appears that the different allelic forms of the Ldh enzyme have differing thermal stabilities (Dimichele and Powers 1991).

An alternative hypothesis explaining the absence of Ldh 100 in introgressed populations of *P. canadensis* is that the Ldh locus is far more closely linked to the diapause locus than has been previously suggested. A close linkage between the "*glaucus*-like" facultative diapause and Ldh 100 would explain why neither traits occurred in these populations. As earlier stated, individuals possessing the facultative diapause gene would be rapidly eliminated from populations in the colder more northern regions of North America.

Primary Research Objectives

- Monitor the dynamics of trait clines for a suite of species diagnostic traits (morphologic, ecological, physiological, and biochemical) in light of changing environmental conditions (ie. average annual thermal unit accumulations). Is the hybrid zone between these two species semi permeable, allowing asymmetric introgression of ecologically significant traits and tightly linked genes?

- Investigate which key trait(s) and ecological parameters are responsible for the historic maintenance of the narrow hybrid zone between these two species. What is the significance of the complete lack of northward introgression of *glaucus*-like Ldh? Is this the result of being more closely linked to the ecologically potent diapause loci?

- Investigate the evolutionary significance of sex-linked traits and co-adapted gene complexes in the process of speciation.

- Utilize this model system to explore the potential for sympatric speciation through the introgression of key sex-linked traits leading to temporal isolation (e.g. "False second flight"). Sufficient temporal isolation could lead to the establishment of reproductive isolation.

- Reevaluate the species status of these two closely related organisms.

Specimen Analysis and Experiments Currently Underway

- Allozyme Data (South / North Manitou 1998 – 2002, Oscoda co., Vermont (2000, 2002)

- Remaining backcross 18006 pupae in diapause (n=27).

- Pc VT – 1st Generation vs. Pc VT – 2nd Generation Emergence Studies (2003) to assess the potential for temporal isolation.

Future Analysis and Experiments

- Continued monitoring and trait cline analysis of key morphological, ecological, biochemical characters in key populations.

- Oviposition analysis of Pc VT EF vs. Pc VT LF lab reared.

- Additional Backcross analysis to determine the frequency with which crossovers occur in part to map and quantitatively determine the distance between Ldh and the diapause loci on the X-chromosome.

- Mitochondrial DNA analysis as additional diagnostic trait to investigate directionality of gene flow across hybrid zone.

- Microsattelite analysis of Pc VT EF vs. Pc VT LF

Masters Thesis 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 research were to examine gene flow between geographically isolated Great Lakes Island subpopulations of swallowtail butterflies, *Papilio canadensis*, using wing trait morphometrics and allozyme electrophoresis. Three Great Lakes island populations and five mainland populations were sampled ranging from Isle Royale in Lake Superior (48° North latitude) to South Manitou Island in Lake Michigan (45° North latitude). There were no significant island versus mainland differences for allozymes (Pgd, Hk, or Ldh) for either Isle Royale or Beaver Island and their respective adjacent mainland populations, but significant differences were detected in wing lengths and black bandwidths. 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 second objective was to further investigate the extent of the *P. glaucus* introgression on and around South Manitou Island. This was accomplished through further analysis of the same morphometric and biochemical characters. In addition, several behavioral (host preference) and physiological (larval survival and growth) traits were included in the analyses. The Tiger Swallowtail butterflies of South Manitou and North Manitou Islands, as well as those of the adjacent mainland, Leelenau Peninsula, exhibit many characteristics making them appear hybrid-like, intermediate between *P. canadensis* and *P. glaucus*. These populations exhibited large forewing lengths, narrow hind wing anal cell black bandwidths, unusual hybrid-like host plant oviposition preferences and larval host plant detoxification abilities for tulip tree (*Liriodendron tulipifera*, of the Mangnoliaceae), as well as possessing relatively high frequencies of two allozyme alleles (Pgd and Hk) diagnostic for *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 (to the Leelenau Peninsula and North / South Manitou Islands; but not to Beaver Island) from that observed inland. This unusual Northward movement of *P. glaucus* genes has created, what can best be characterized as an isolated "hybrid swarm" on and around the Manitou Islands.