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THE INFLUENCE OF FEMALE WING COLOR VARIATION ON MALE MATE CHOICE IN TIGER SWALLOWTAIL BUTTERFLIES (*PAPILIO* SPP.)

presented by

Matthew L. Aardema

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Major Professor's Signature

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Date

THE INFLUENCE OF FEMALE WING COLOR VARIATION ON MALE MATE CHOICE IN TIGER SWALLOWTAIL BUTTERFLIES (*PAPILIO* SPP.)

By

Matthew L. Aardema

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ABSTRACT

THE INFLUENCE OF FEMALE WING COLOR VARIATION ON MALE MATE CHOICE IN TIGER SWALLOWTAIL BUTTERFLIES (*PAPILIO* SPP.)

By

Matthew L. Aardema

Variation both within and between populations is an important component of the speciation process. This variation can be found in all biological arenas and includes ecological variation, developmental variation and variation in sexual characteristics. In Lepidoptera, this last source of variation has had an important role in producing divergence between populations. It has been shown that males of the eastern tiger swallowtail, *Papilio glaucus*, and the Canadian tiger swallowtail, *P. canadensis,* both prefer to mate with female *P. glaucus* in two-choice field experiments. This suggests that there is a species difference between females of the two species that is recognizable to males. One possible difference is the amount of blue scaling on the dorsal wing surface of females. *P. glaucus* females have relatively more area of their wing surface covered by this blue coloration than do *P. canadensis* females. However, my experiments to determine the importance this blue coloration has for males selecting a mate suggests that its influence is minimal and hence the difference in blue coloration seen between *P. glaucus* and *P. canadensis* is unlikely to explain differences in male mate choice. Other explanations for asymmetrical male mate choice may be differences in body size and/or yellow coloration in females of these two species. Although quantified, these were not experimentally tested to determine their importance in male mate selection.

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CHAPTER 1:

INTRODUCTION

It has been argued that variation within and between populations is one of the most interesting, yet overlooked aspects of biological diversity (Mayr, 2005). Subtle differences among individuals within and between populations may lead to remarkable divergences and biological radiations (Schluter, 2000). For example, it has been suggested that differences in beak width and depth helped to generate the diversity of Darwin's finches on the Galapagos Islands (Grant, 1986), morphological variation and the influence this has on pollinator complexes lead to the diversification of orchids (Tremblay *et al.*, 2005), and variation in teeth shape lead to ungulate mammalian diversification (Jernvall *et al.*, 1996).

Among most lepidopteran groups important ecological (Ayres and Scriber, 1994; Bossart and Scriber, 1995, Mallet and Joron, 1999), developmental (Beldade and Brakefield, 2002), and sexual variation (Ellers and Boggs, 2003) is common within and between extant populations and such variation likely played a large role in the historical diversification of this clade (Boggs *et al.,* 2003 and chapters within). Several factors contribute to the high degree of population variation observed in butterflies including a complex life history (Beck and Fiedler, 2009) and rapid generation times (Thomas *et al.*, 2010).

Variation in traits related to mating and reproduction has been especially important in butterfly speciation (Chamberlin *et al.*, 2009). In Lepidoptera, the male is the homogametic sex and the sex chromosomes are designated Z [=X] and W [=Y] respectively. This sex determination system allows for the generation of a relatively large amount of sexual dimorphism in many species, possibly because genes that generate 'preference' for particular sexual traits can be maintained on the W chromosome in females, without the disadvantages of also being found in males that have the potentially costly trait (Hastings, 1994). The presence of such sexual dimorphism (specifically variation in secondary sexual characteristics and preferences for these characteristics) can lead to differential selection and assortative mating (Naisbit *et al.*, 2001) when subsets of males and females preferentially mate with one another at higher frequencies than they mate with other individuals of the population. This process can lead to the formation of new species.

The Tiger Swallowtail System

Within the swallowtail family, Papilionidae, there are between 475 to 563 species worldwide (Scriber, 1995). Of these, the tiger swallowtail clade comprises between 5 and 8 species in North America. In the eastern United States and Canada, two species occur with a parapatric distribution (Scriber, 1996a). The Canadian tiger swallowtail, *Papilio canadensis*, is found from central Alaska, throughout much of Canada, south into northern Minnesota, Wisconsin and Michigan, and east into the New England States (Fig. 1; Scriber, 1996a). The range of *P. canadensis* overlaps with that of the eastern tiger swallowtail, *P. glaucus,* in a narrow zone of hybridization through central Wisconsin, Michigan and New York, and along the northern Appalachian mountain range extending

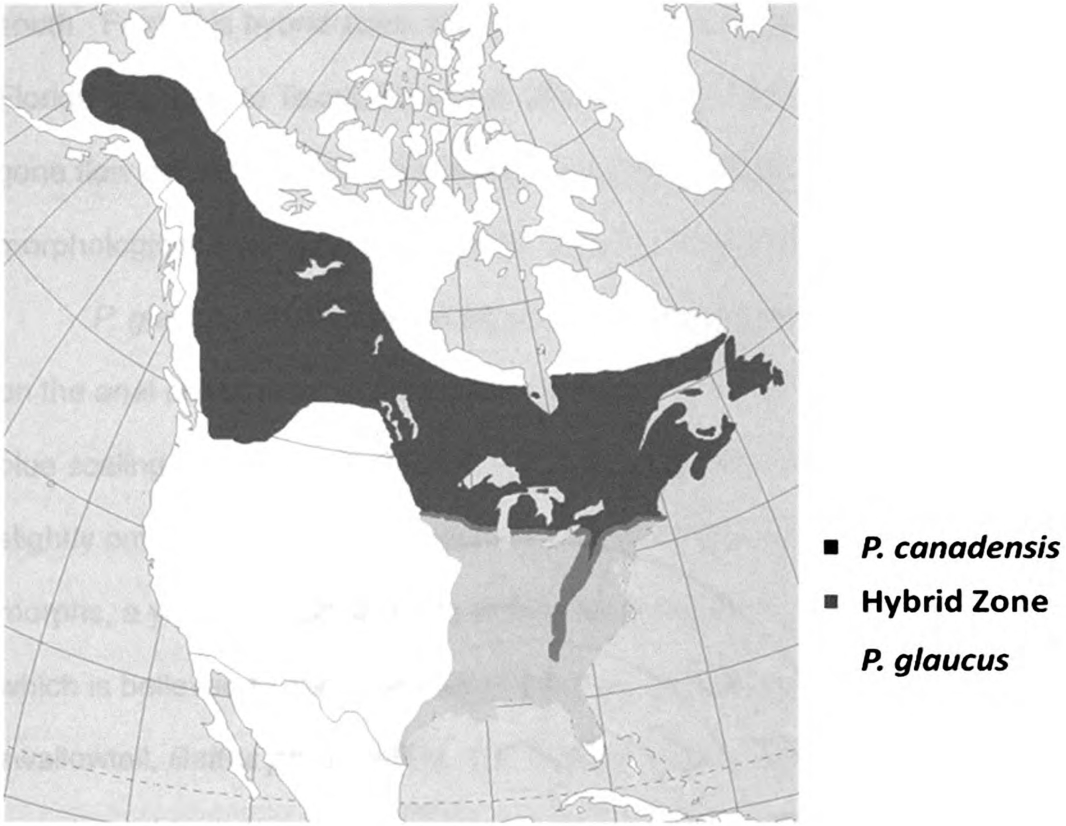

Figure 1.1. Distribution map of *P. canadensis, P. glaucus,* and areas of hybridization between them in North America.

south. From this hybrid zone, the range of *P. glaucus* reaches south to central Florida and west to Texas, Arkansas, Missouri and Illinois. Despite continued gene flow between these two species, they exhibit many important morphological, ecological and genetic differences (summarized in Table 1).

P. glaucus individuals are larger and have a relatively narrower black band on the anal cell of their hind wings (Luebke *et al.*, 1988). Females have more blue scaling on the dorsal side of their hindwings (see Ch. 2) which extends slightly onto the forewing. Additionally, female *P. glaucus* occur as two color morphs, a yellow morph which is similar in coloration to males, or a dark morph which is believed to mimic the black and blue coloration of the male pipevine swallowtail, *Battus philenor* (Fig. 1.2; Brower, 1958). The frequency of these two morphs within a population appears to be correlated with the density of *B. philenor* occurring congruently (Brower and Brower, 1962).

In contrast to *P. glaucus*, individual *P. canadensis* are generally smaller and have a relatively wider black band. Females have less overall dorsal blue coloration which does not extend onto the upper wing. Female dark morphs do not exist in pure *P. canadensis* populations due to the absence of a melanism gene (b+, found on the W- chromosome and maternally inherited) and the additional presence of a suppresser gene (found on the Z- chromosome and paternally inherited in females; Scriber *et al.* 1996).

The two most important ecological differences occur in diapause induction (and corresponding voltinism patterns; Rockey *et al.*, 1987), and larval host-use

Table 1.1. Summary of morphological, ecological and genetic differences between *P. glaucus* and *P. canadensis*

TRAIT	*P. CANADENSIS*	*P. GLAUCUS*	REFERENCE
Morphological			
Size	Smaller (35 - 50 mm upperwing length)	Larger (50 - 70 mm upperwing length)	Luebke *et al.*, 1988; Aardema Pers. Obs.
Blackband Width	Wider (50 - 90% of anal cell width)	Narrower (10 - 50% of anal cell width)	Luebke *et al.*, 1988; Aardema Pers. Obs.
Female Blue Coloration Extends onto Upperwings	No	Yes	Aardema, Pers. Obs.
Female Coloration	Only Yellow Morph	Yellow or Dark Morph	*Hagen and Scriber, 1989;* Scriber *et al.*, 1996
Ecological			
Diapause Induction	Obligate	Facultative	Rockey *et al.*, 1987
Larval Host Use	Very polyphagous, but tulip tree is toxic	Very polyphagous, but quaking aspen is toxic	Scriber, 1988
Genetic			
Mitochondrial COI and COII genes	19 Fixed Differences		Andolfatto *et al.*, 2003; Putnam *et al*, 2007
Mimicry Genes	Absence of Dark Morph enabler gene on W chromosome and Presence of Dark Morph Suppresor on Z chromosome	Presence of Dark Morph enabler gene on W chromosome and absence of Dark Morph Suppresor on Z chromosome	*Hagen and Scriber, 1989*
Z-linked Allozyme Pgd	-125/-80/-150	-100 or -50	*Hagen and Scriber, 1991*
Z-linked Allozyme Ldh	-80 or -40	-100	*Hagen and Scriber, 1991*
Autosomal Allozyme Hk	-110	-90 or -100	*Hagen and Scriber, 1991*
AFLP Analysis	Many Species Level Differences		Winter and Porter, 2009

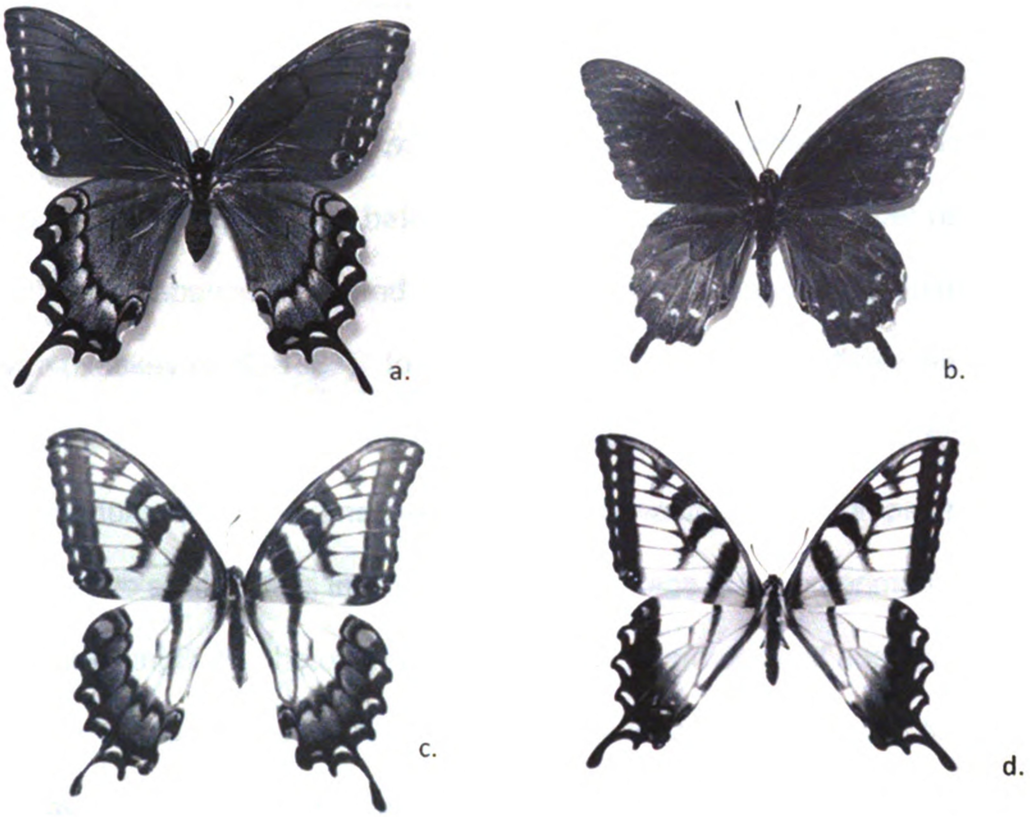

Figure 1.2. a.) Dark morph, *P. glaucus* female; believed to mimic the distasteful pipe-vine swallowtail, *Battus philenor* b.) *Battus philenor* male c.) Yellow morph, *P. glaucus* female d.) *P. glaucus* male

abilities (Scriber, 1988). *P. canadensis* are obligatory diapausing and only complete only one generation per year (univoltine).

They overwinter in the pupal stage and must experience a prolonged period of cold temperatures before they will break diapause. Under natural conditions, diapause is entered regardless of the photoperiod experienced by the larvae (Rockey *et al.*, 1987). In contrast, *P. glaucus* is facultatively diapausing and can either enter a diapause stage like *P. canadensis*, or else directly develop into an adult within the same year that larval development is completed. Which of these two strategies is initiated in the pupal stage is based primarily on light cues (day length or 'photoperiod'), although temperature and host quality may also play a role (Rockey *et al.*, 1987). All populations of *P. glaucus* complete at least two generations per season (bivoltine), and in some locations (e.g. southern Georgia and Florida), three or more generations may be possible (multivoltine; Rockey *et al.*, 1987, Scriber *et al.*, 2008).

Tiger swallowtail butterflies are among the most polyphagous of all butterflies with regards to larval host use abilities. Larvae can utilize a large number of deciduous tree species from 10 disparate families (Scriber *et al.*, 1982; Scriber, 1988). These include black cherry (*Prunus serotina*), ash (*Fraxinus* spp.) and hop tree (*Ptelea trifoliata*). However, adult female ovipositional preferences tend to be narrower. One of the most preferred ovipositional hosts of *P. glaucus* is tulip tree (*Liriodendron tulipifera*). Larvae of *P. glaucus* can utilize tulip tree effectively and grow and survive on it similar to other good host species (Mercader *et al.*, 2009). In contrast, tulip tree is toxic to *P.*

canadensis larvae and causes complete mortality in larval families fed this species (Scriber *et al.*, 1982). Conversely, one of the preferred ovipositional hosts of *P. canadensis* is quaking aspen (*Populus tremuloides*) which is toxic to larvae of *P. glaucus* (Scriber *et al.*, 1989). Interestingly, in ovipositional assays *P. canadensis* females will often lay a high proportion of their eggs on tulip tree (Mercader and Scriber, 2007; 2008a). This maladaptive tendency is unlikely to have any negative impact on natural populations as the range of tulip tree does not overlap with *P. canadensis* (Mercader and Scriber, 2007). In ovipositional assays, *P. glaucus* females lay very few eggs on quaking aspen (Mercader and Scriber, 2007; 2008a).

Lastly, there are many genetic differences between the two species. These include fixed nucleotide differences in the COI and COII mitochondrial genes (Andolfatto *et al.*, 2003) and in several genes found on the Z-chromosome (Putnam *et al.*, 2007). Additionally, AFLP markers exhibit clear species diagnostic differences (Winter and Porter, 2009). There are fixed species differences in the Z-linked allozymes Pgd and Ldh, as well as the autosomal Hk (Hagen and Scriber, 1989; 1991). Finally, the aforementioned enabler/suppressor complex of genes which facilitates the presence of dark morph females in *P. glaucus* populations may be considered an important genetic difference (Scriber *et al.,* 1996a).

Local adaptation between populations of each species has been observed (Ayres and Scriber, 1994; Bossart and Scriber, 1995; Scriber 1996b; 2002), indicating that in some circumstances selection pressures can be strong enough to overcome the homogenizing tendencies of migration and gene flow. However, between *P. glaucus* and *P. canadensis*, historical hybridization has clearly occurred, facilitating the exchange of genomic regions and producing hybrid populations (Luebke and Scriber, 1988; Scriber, 1990a; Stump *et al.*, 2003; Scriber and Ording, 2005, Kunte *et al.*, In Prep). This is an interesting observation as hybridization between *P. glaucus* and *P. canadensis* results in both direct fitness reductions (in part due to a Haldane effect; Hagen and Scriber, 1995) and extrinsic fitness reductions that include failure to enter diapause in areas at northern latitudes where it is impossible to complete two generations in one season (Ritland and Scriber, 1985; Hagen and Scriber, 1989), and the initiation of diapause after one generation (obligate diapause) in more southerly regions where the time spent in this stage during the warmest parts of the year incurs metabolic costs which reduce survival and fitness (e.g. fecundity, etc.; Scriber *et al.*, 2002; Mercader and Scriber, 2008b).

There are several explanations to account for incomplete reproductive isolation and continued gene flow between *P. glaucus* and *P. canadensis*. First, hybridization occurring directly between pure parental genotypes is likely rare (Donovan and Scriber, 2003). Gene flow more likely occurs along a gradient of backcrossed individuals throughout the hybrid zone from pure *P. glaucus* south of the zone to pure *P. canadensis* north of the zone (Hagen, 1990). This means that direct costs to hybridization (e.g. reduced viability) are diminished and may not be strong enough to generate significant reinforcement to prevent hybridization. Furthermore, extrinsic costs to hybridization are further reduced as specific genetic regions that cause strong fitness reductions are quickly purged from populations (e.g. facultative diapause in the northern parts of the hybrid zone is 'suicidal'; Martinsen *et* al., 2001; Scriber *et al*, 2008).

Another possible explanation for the presence of continued gene flow is an asymmetrical mating preference observed in male tiger swallowtails. In two-choice mate preference assays, Deering and Scriber (2002) observed that males of both *P. glaucus* and *P. canadensis* strongly preferred to mate with *P. glaucus* females. This suggests that within and close to the hybrid zone, *P. glaucus* males may exhibit reduced inclinations to mate with *P. canadensis* and possibly hybrid females. However, it is also probable that male *P. canadensis* would readily mate with any *P. glaucus* females they encounter and likely hybrid females as well. There are two caveats to this suggestion. First, Deering and Scriber did not examine male mating preferences near or within the hybrid zone and as seen in other hybrid zone systems (e.g. Peterson *et al.*, 2005) mating preferences may be weaker or stronger depending on proximity to areas where hybridization occurs. Secondly, as the cues that resulted in males of both species preferring *P. glaucus* females are unknown (see below), we have no knowledge of how these cues may be inherited and thus how males of either species would respond to hybrids of various genetic backgrounds.

Differential preference for specific phenotypes could manifest itself as complete refusal to engage a female or relatively less time/energy spent in pursuit of a female who initially rejects a male's advances (Krebs, 1988; Aardema, pers. obs.). The interspecific dynamic in male mate preference may, in part, explain continued hybridization between the two species, as well as the specific ecological and genetic characteristics of several unique hybrid populations that have been observed (Scriber and Ording, 2005, Mercader *et al.*, 2008; Kunte *et al*, In Prep.).

Male Mate Choice in Tiger Swallowtails

Male mate choice is defined as a differential response of individual males to conspecific, reproductively mature females which vary in some quality recognizable to the male (Bonduriansky, 2001). Male mate choice is expected to arise in systems where the male contributes resources to the formation of offspring aside from sperm (Bonduriansky, 2001). Additionally, when there is a large amount of variance in female quality, males are also predicted to exhibit some degree of discrimination.

When male tiger swallowtails mate, they pass a large packet of nutrients and sperm (known as a spermatophore) to the female. To produce this packet, males engage in a behavior called puddling, which involves the uptake of moisture and nutrients from wet substrate, dung, carrion, and other similar resources (Arms *et al.*, 1974). Male tiger swallowtail butterflies are frequent puddlers, regularly accumulating in large numbers at high quality puddling locations (Arms *et* al., 1974; Scriber and Ayres, 1988; Otis *et al.*, 2006). Male *Papilio* are severely limited in their reproductive ability if they are unable to intake nutrients (Lederhouse *et al.*, 1990). The need of males to puddle in order to acquire sufficient resources to form a spermatophore likely limits the number of successful copulations males can engage in during their lifetime (Lederhouse *et al.*, 1990; Bissoondath and Wiklund, 1996; Lewis and Wedell, 2007). Furthermore, puddling puts males in danger from predation (Morris, 1953; Rawson and Bellinger, 1953). Finally, the first spermatophore of a male is significantly larger than subsequent spermatophores (Lederhouse *et al.*, 1990).

The spermatophore represents a significant contribution of time, energy, and resources (Arms *et al.*, 1974). As encountered females will vary in the number of eggs they have available (both absolutely and due to previous oviposition), it is perhaps not surprising that males exhibit mating preferences. Examinations of spermatophore number have revealed that in most circumstances a single mating is likely sufficient to fertilize the entirety of a female's eggs (Levin, 1973; Lederhouse and Scriber, 1987). However, when initial spermatophores are small, subsequent matings may be necessary to ensure all eggs are fertilized (Lederhouse and Scriber, 1987). Additional matings may also provide important nutrients for female somatic maintenance. Accordingly, females frequently solicit male matings (Krebs, 1988). .

The high cost of a spermatophore creates a strong impetus for males to exhibit some degree of female mate choice. Furthermore, mating studies also lend credence to the assertion that males exhibit mate choice and preferences (Brower, 1959; Deering and Scriber, 2002). The female characteristics that *Papilio* males use to select conspecific mates are presently unknown. Nor is it known whether traits being assessed in intraspecific encounters are the same responsible for the interspecific mating preferences exhibited by *P. canadensis* males for *P. glaucus* females. However, it is reasonable to suspect that the trait (or traits) used by males to judge females in intraspecific encounters are the same that generate asymmetrical, interspecies preferences and that this trait generally exists in a more 'preferred' state in *P. glaucus* females.

Research Objectives

Given the asymmetrical mating preferences exhibited by male tiger swallowtail butterflies, I postulate that a single morphological trait or small number of traits differs between *P. glaucus* and *P. canadensis* and is recognizable and distinguishable by male tiger swallowtails. This trait is likely female limited. I also predict that variation in some quality of this trait will result in a greater or less likelihood of males attempting to copulate. This trait may either be directly correlated with fitness, or may simply be a mate recognition cue that allows males to recognize females of their own species (or a species that is very similar in appearance). In this case, interspecific preferences may be the result of this trait eliciting a stronger mate recognition response.

CHAPTER 2:

VARIATION IN WING COLORATION BETWEEN *PAPILIO CANADENSIS* AND *P. GLAUCUS* POPULATIONS

Introduction

In the context of evolutionary processes, large amounts of variation in morphological characteristics exist and persist for several different reasons. One explanation for the maintenance of such variation is an absence of consistent, strong stabilizing selection on a trait (Heegaard, 1997; Kotanen and Bergelson, 2000). Selection that acts in a relatively regular fashion will generally narrow a trait's phenotypic variance by reducing genetic variation contained within individuals that are less fit than population conspecifics (Kaufman *et al.*, 1977). Conversely, an absence of some regular selection permits a trait to vary to the extent genetic variability allows (Hunt *et al.*, 2007). Environmental influences, polygenic effects, and differences in gene regulation can all increase phenotypic variation in a trait (Turelli and Barton, 2004).

Another cause of extensive morphological variation is the presence of multiple, divergent selection pressures that act on a trait, preventing stabilization and the reduction of variance (Grant, 1986). This may happen *within* a population, causing the variance of a trait to remain broad and preventing the narrowing of allelic diversity (Saint-Laurent *et al.,* 2003, Yeaman and Jarvis, 2006), or it may be that specific pressures differ in nature and intensity *between* populations, creating local adaptation with limited variation within specific areas, but wide variation within the species as a whole (Linhart and Grant, 1996).

Depending on their extent, migration and gene flow will impact tendencies for local adaptation substantially (Yeaman and Jarvis, 2006).

Tiger swallowtails in the eastern USA are known to vary in many ecological and morphological traits within populations (Luebke *et al.*, 1988; Hagen, 1990; Mercader and Scriber, 2007; Mercader *et al.*, 2009), between different populations of the same species (Hagen, 1990; Scriber, 1996b; 2002; Kunte *et al.*, In Prep.), and between different species (Scriber *et al.*, 1989; Stump *et al.*, 2003; Kunte *et al.*, In Prep.). Much of this variation is linked to specific climatic conditions in the respective geographical ranges of these species and populations. Climate and temperature influences variation either directly due to selection created by different thermal tolerances (Mercader and Scriber, 2008b), or else indirectly as mediated through host-plant ranges and phenologies (Scriber and Ording, 2005). This selection is particularly important for maintaining the species boundary between *P. canadensis* and *P. glaucus,* mostly through a post-zygotic reduction in survival of hybrid offspring outside the hybrid zone.

Male and female mating preferences may also have importance for maintaining these species' identities in addition to influencing hybrid zone introgression patterns (Stump *et* al., 2003; Scriber and Ording, 2005; Kunte *et al.,* In Prep). Variable traits that are important for either mate recognition and/or mate preference either within or between these species remain unknown, although evidence suggests this may be another important and possibly non-thermally linked area of variation (Deering and Scriber, 2002). Male mating preferences are particularly intriguing as tiger swallowtail males have been shown to exhibit choice for both conspecifics (Brower, 1959; Deering and Scriber, 2002) and heterospecifics (Deering and Scriber, 2002), and also for females that appear 'young' versus those that appear 'old' (as indicated by scale loss; Scriber and Aardema, unpublished data). Furthermore, males will reject potential conspecific mates outright (Krebs, 1988; Aardema, pers. obs.), possible because they appear older and are likely to have less eggs or possibly because of some perception of genetic inferiority. This further indicates that male mate preferences may be a fruitful area of investigation for understanding potential pre-zygotic isolating mechanisms between tiger swallowtail butterflies.

The color vision of butterflies in the genus *Papilio* has a wide spectral range that extends down into the UV (300 – 400 nm) and up into the infrared (700 – 800 nm) (Briscoe, 2000). Additionally, these butterflies may be among the best of any organism at discriminating between similar shades of color (Koshitaka *et al.*, 2008). Visual assessment of females by males is common in many Lepidopteran species (Bonduriansky, 2001). These facts suggest that a visual cue or cues may be important in tiger swallowtail mate recognition and/or mate assessment. In particular, a consistent difference in some component of coloration between *P. canadensis* and *P. glaucus* females may explain previously observed asymmetrical male mating preferences (Deering and Scriber, 2002).

The extent of dorsal blue coloration has been noted to appear qualitatively different in the two species, with *P. glaucus* females seeming to have substantially more of their wing surface covered by blue scaling (Deering and

Scriber, 2002). Additionally, although both males and females have some dorsal blue scaling, *extensive* blue coloration appears limited to females (Scriber, 1990b; Aardema, pers. obs.). Both female color morphs of *P. glaucus* have this coloration, making it a potential 'common recognition characteristic' that may aid males in recognizing both color morphs as conspecific females (Platt *et al.*, 1984; Hereau and Scriber, 2002). In populations where both color morphs are present, individual males do recognize females of each morph as potential mates and will attempt to copulate with individuals of both colors (Aardema, pers. obs.).

To locate females, males generally patrol forest edges along streams, roadways, power cuts and other, similar linear paths. They fly above nectar sources along these paths looking for conspecifics. It is believed that yellow coloration and movement provide initial recognition cues, as males will engage both males and females when first encountered, generally approaching from above and behind (Aardema, pers. obs.). After initial contact, males usually recognize other males and break off contact quickly. Conversely, if a female is encountered the male will pursue her until she either accepts his attempts to copulate or else loses him via rapid flight through the undergrowth (Deering and Scriber, 2002). Although some subtly differences in this behavior between *P. canadensis* and *P. glaucus* males have been noted (Deering and Scriber, 2002), general patterns of mate location are similar between the two species.

This type of mate location strategy suggests that a visually-based, dorsally located, gender-specific cue such as blue coloration is highly likely to aid males in locating and recognizing females. A female with reduced blue coloration may appear ‘male-like’ and receive reduced copulation attempts (and a potentially corresponding reduced fitness) relative to females with more extensive blue. Additionally, as the spermatophore is a valuable resource and males seemingly exhibit some degree of mate selection, it is possible that this blue coloration may be linked to female fitness in some presently unknown way.

To determine whether the relative amount of blue coloration on the dorsal wing surface differs between female *P. glaucus* and *P. canadensis* (independent of overall size), I quantified the relative extent of blue coloration in females and males of both species on both the ventral and dorsal side of the wings. Based on my qualitative observations, I predicted that the difference between males and females of both species would be significant on the dorsal side of the wings, but not the ventral side. I also postulated that female *P. glaucus* and *P. canadensis* will be significantly different in dorsal coloration. I predicted that in no possible comparison (between sexes or species), would ventral blue coloration differ significantly.

Additionally, because ultraviolet color is important in many lepidopteran systems for mate recognition and selection (Brunton and Majerus, 1995), I measured wing regions of blue, yellow and black coloration for the presence or absence of UV reflectance. Given that these *Papilio* butterflies can see well into the UV spectrum, I hypothesized that the blue coloration would reflect a relatively large amount of UV light, the yellow would reflect a relatively small amount of UV light and the black would reflect no UV light. A finding of UV reflectance would suggest potentially fruitful avenues of future inquiry.

Materials and Methods

Samples

To analyze differences in blue coloration between populations and species of tiger swallowtail butterflies I collected pristine or near pristine (little or no wing damage and scale loss) wild females from populations in Fairbanks-North Star Co., Alaska (n=11), Charlevoix Co., Michigan (n=12), Jackson and Gallia Cos., Ohio (yellow morph: n=8, dark morph: n=9) and Levy Co., Florida (yellow morph: n=15, dark morph: n=10). I also obtained females from two additional populations, one in Bennigton Co., Vermont (n=7) and one in Habersham and Rabun Cos., Georgia (yellow morph: n=4, dark morphs: n=2). Collectively these six populations span the latitudinal range of both *P. glaucus* and *P. canadensis* (Fig. 2.1). Males from each of these populations were also collected for female/male comparisons (Alaska= 10; Michigan=12; Ohio= 12; Florida= 11; Vermont= 9; Georgia= 3).

Wing Preparation and Photography

Using a fine bladed scissors, I carefully removed all four wings from each specimen at the wing base where it attaches to the thorax. After removal, wings were kept in glassine envelopes until photographed. I used an Olympus FE-310 (8.0 megapixels) digital camera to photograph both sides of minimally one hind wing and one forewing from each specimen (Fig. 2.2a). In some instances all four wings were in pristine condition and I was able to take two photographs, one of each wing set. I generally tried to match wings from the same side of the body, but occasionally this was not possible because of damage or scale loss to two separate wings.

Analysis of Relative Blue Area

To analyze these photographs, I used the imaging program ImageJ (Abramoff *et al.*, 2004) with standard tools to measure the total area of the two wings (Figure 2.2b). I then used the Threshold_Colour plugin (Landini, 2010) to remove all coloration in the image except blue and then the standard ImageJ tools to measure the area of this coloration (Figure 2.2c). For both measurements, area was calculated as the number of pixels per unit measurement. With the measurements of total wing area and blue area, I was able to calculate a relative proportion of blue coloration. This allowed me to make comparisons of blue area that were independent of the general size differences between *P. glaucus* and *P. canadensis.* When two images were taken for the same butterfly, the mean of their relative blues areas was used in analysis.

Ultraviolet Examination

I first assessed the presence or absence of ultraviolet coloration qualitatively by using a UV bypass filter attached to a Nikon Coolpix 990 (3.34 megapixels) digital camera. With this combination of filer and camera, I photographed six fore- and hind wing pairs from *P. glaucus* females in a darkened room (to reduce full spectrum light) with a circular black light for illumination (Acorn, 2002). Using this technique I could visualize areas that were UV reflective and those that were UV absorbent.

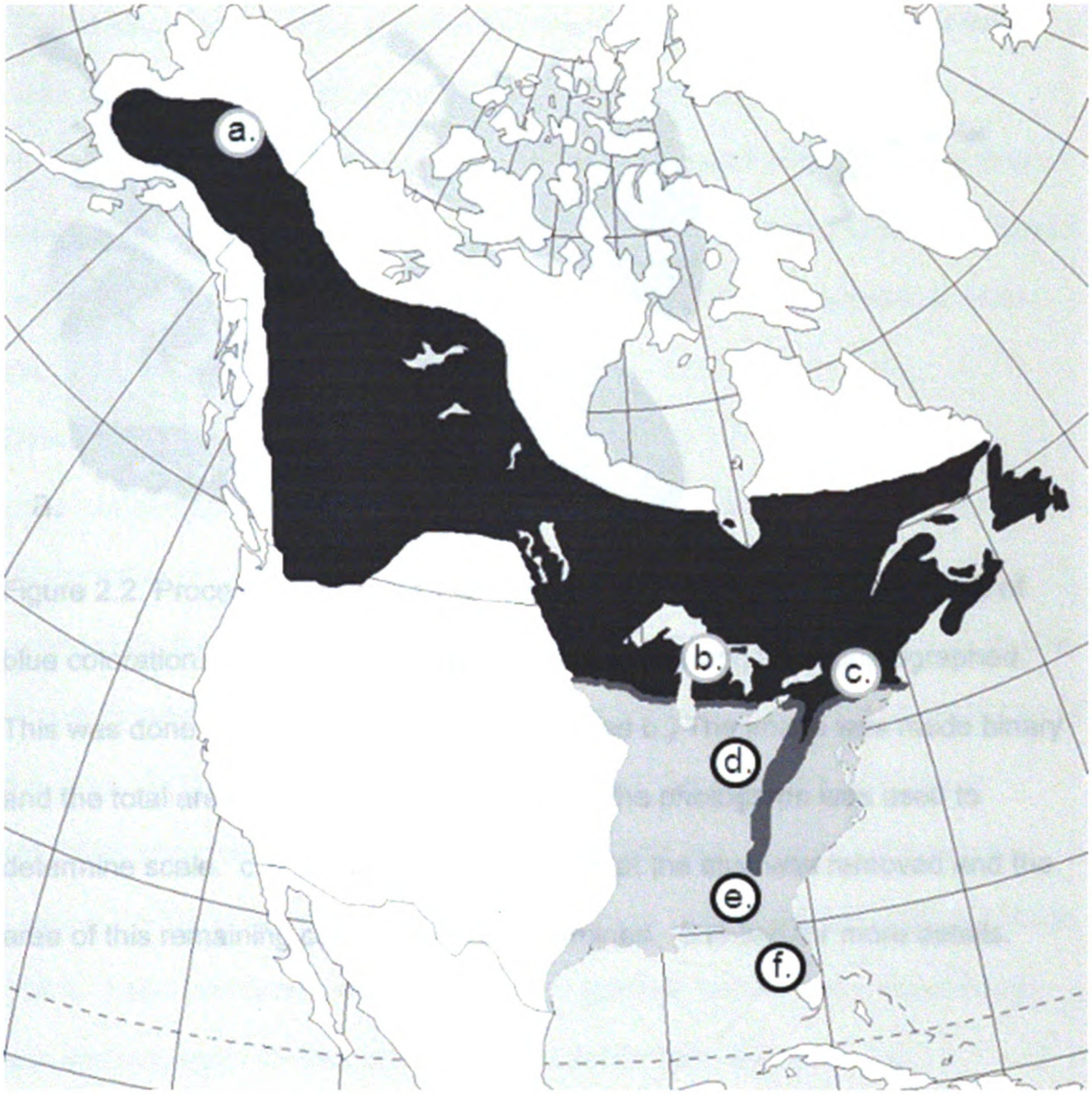

Figure 2.1. Locations of sample populations for blue color analysis. Gray circles represent *P. canadensis* populations and black circles represent *P. glaucus* populations. a.) Fairbanks-North Star Co., Alaska b.) Charlevoix Co., Michigan c.) Bennigton Co., Vermont d.) Jackson and Gallia Cos., Ohio e.) Habersham and Rabun Cos., Georgia f.) Levy Co., Florida

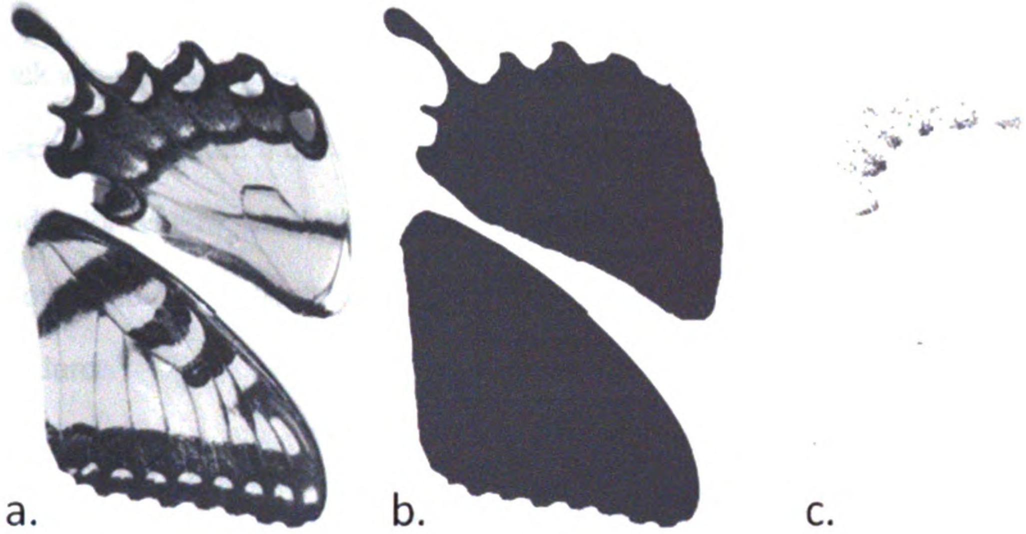

Figure 2.2. Process of wing analysis to determine total wing area and area of blue coloration. a.) First, one forewing and one hindwing were photographed. This was done for both dorsal and ventral sides b.) The image was made binary and the total area was determined. A ruler in the photograph was used to determine scale. c.) Lastly, all coloration except the blue was removed and the area of this remaining coloration was determined. See text for more details.

I also measured the spectral reflectance signature of blue, yellow and black wing areas using an Ocean Optics s2000 miniature fiber optic spectrophotometer. I cut three roughly 1 cm^2 squares of wing that had a single solid color (yellow, black or blue) from the pristine wings of four Levy Co., Florida yellow females. Reflectance of color was measured in comparison to a white standard.

Data Analysis

All statistical analysis was performed using the statistical package R (Abramoff *et* al., 2004). I made overall mean comparisons between male *P. canadensis*, female *P. canadensis*, male *P. glaucus*, and yellow- and dark morph female *P. glaucus* for the relative blue area of both the dorsal and ventral sides of the wing. To do this I used either a standard one-way analysis of variance (ANOVA) when variances were homogenous (as determined by Levene's test; Levene, 1960), or, when variances were unequal, I used Welch's ANOVA (Welch, 1951). I also performed ANOVAs comparing males and females of individual populations, again for both dorsal and ventral relative blue wing area. I performed comparisons for each of the populations from the above analyses using pairwise t tests with adjusted p values using the Bonferroni step-down correction method develop by Holm (Holm, 1979). This correction gives control over the family wise error, but is less conservative than the simple Bonferroni correction.

Finding a statistical difference for relative blue area in species comparisons, I performed a one-way analysis of covariance (ANCOVA) comparing the total wing area and the area of blue with species as a co-factor to determine if observed differences in blue were an artifact of size differences between *P. canadensis* and *P. glaucus* or rather were indicative of a fundamental allometric difference between the two species. This would be indicated by statistically significant differences in either the slope and/or y-intercept. I compared three models: a simple model without species as a cofactor, an additive model with species as a cofactor and a multiplicative model, again with species as a cofactor. Models were compared using adjusted coefficient of determination (Adj. R^2), the Akaike information criterion (AIC; Akaike, 1978) and the Schwarz Bayesian Information Criterion (BIC; Schwarz, 1978). For all statistical tests, an alpha value of 0.05 was considered significant. No statistical tests were performed for ultraviolet coloration, but rather a qualitative assessment was made.

Results

For all comparisons, variances were homogenous except for the dorsal comparison of relative blue area between species/sex results. There was a significant difference in dorsal blue area [$F(4,57.11)=32.05$, $p < 0.001$]. Specifically, both yellow-morph and dark-morph *P. glaucus* females differed significantly from female *P. canadensis,* male *P. canadensis* and male *P. glaucus* (Fig. 2.3). No other sex or species comparison differed significantly. There were no significant differences in ventral relative blue area [$F(4,128)=1.71$, $p=0.151$]; (Fig. 2.3).

Between populations of female *P. canadensis* and female yellow-morph *P. glaucus* there were significant differences between the mean relative dorsal blue area [F(4,48)=23.235, p<0.001]. In concordance with the species/sex result, both *P. glaucus* populations differed from the three *P. canadensis* populations (Fig. 2.4). No female *P. canadensis* populations differed significantly from one another, nor did the three *P. glaucus* populations differ from one another. There were no significant differences in ventral blue coloration between any population of female *P. canadensis* and female yellow-morph *P. glaucus* [F(4,48)=1.724, p=0.160]; (Fig. 2.4)

Variance between the three populations of dark-morph female *P. glaucus* was homogenous for dorsal blue, and the means did not differ significantly between the populations [F(2,18)=0.735,p=0.494]. Variance between the two populations for ventral blue was homogeneous and an ANOVA revealed no difference in means as well [F(2,18)=0.276,p=0.762).

Surprisingly, there were differences in mean male dorsal blue between populations [F(4,49)=7.590, p<0.001]. However, unlike in females this variation did not occur in relation to species (Fig. 2.5). Males from the Vermont population (*P. canadensis*) differed from the Alaska and Michigan populations (also *P. canadensis*), and Ohio (*P. glaucus*) likewise differed from Alaska and Michigan. Also surprisingly, male ventral blue coloration differed significantly between some populations

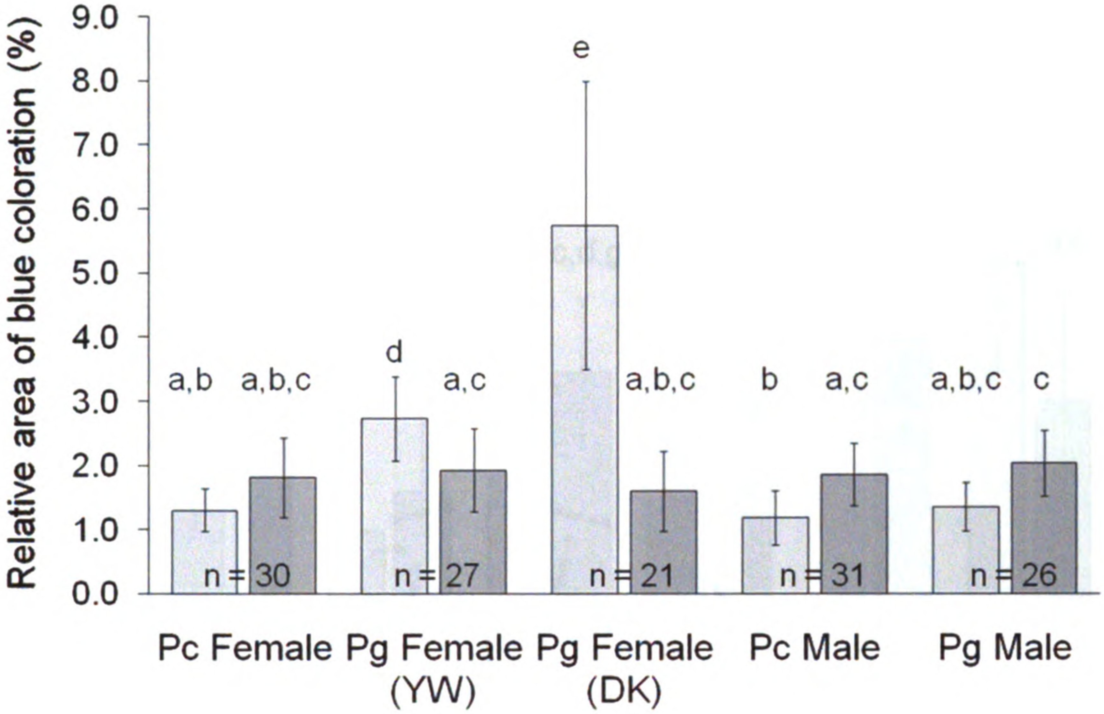

Figure 2.3. Mean relative blue area ± SD for all species/sex/color morph combinations. Pc : *P. canadensis*, Pg : *P. glaucus*, YW : yellow morph females and DK : dark morph females. For each combination, the light grey bar shows the mean blue coloration on the dorsal wing surface and the dark grey bar shows the mean blue coloration on the ventral wing surface. Bars with the same letter are not significantly different at the $p<0.05$ level (pairwise t test with Holm's correction).

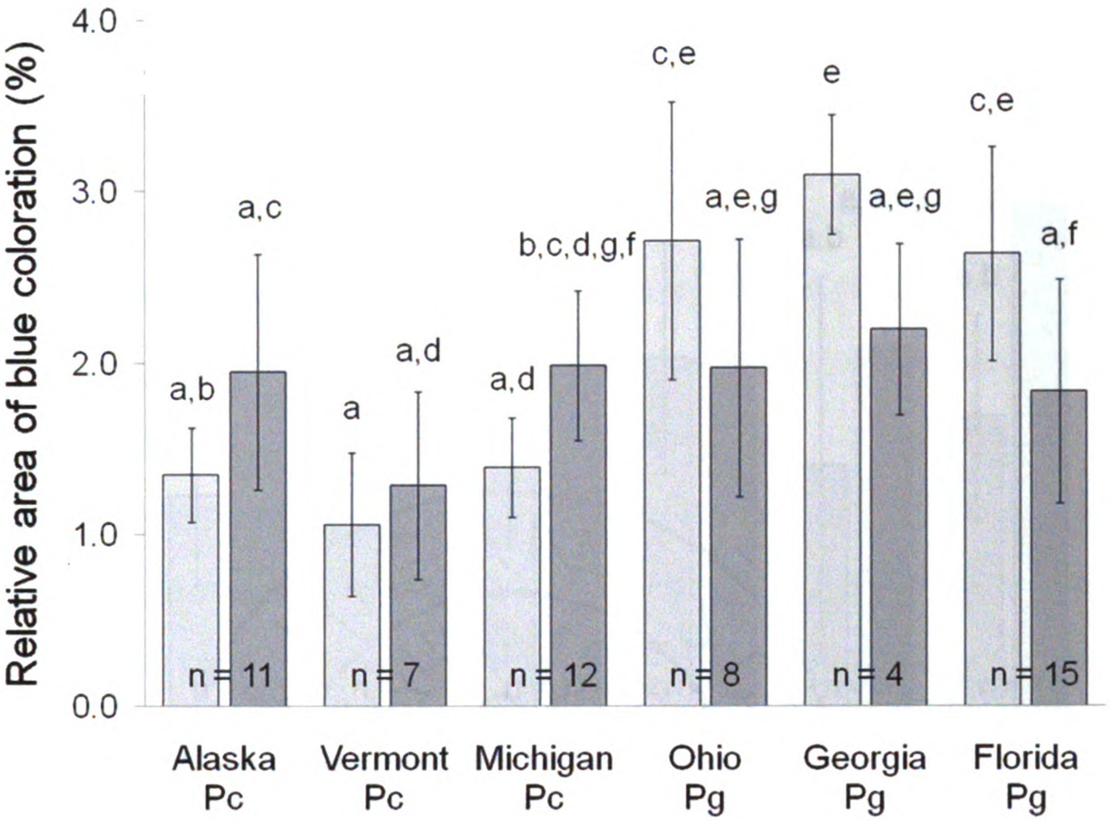

Figure 2.4. Mean relative blue area ± SD for all female *P. canadensis* (Pc) and yellow morph *P. glaucus* (Pg). For each combination, the light grey bar shows the mean blue coloration on the dorsal wing surface and the dark grey bar shows the mean blue coloration on the ventral wing surface. Bars with the same letter are not significantly different at the p<0.05 level (pairwise t test with Holm's correction).

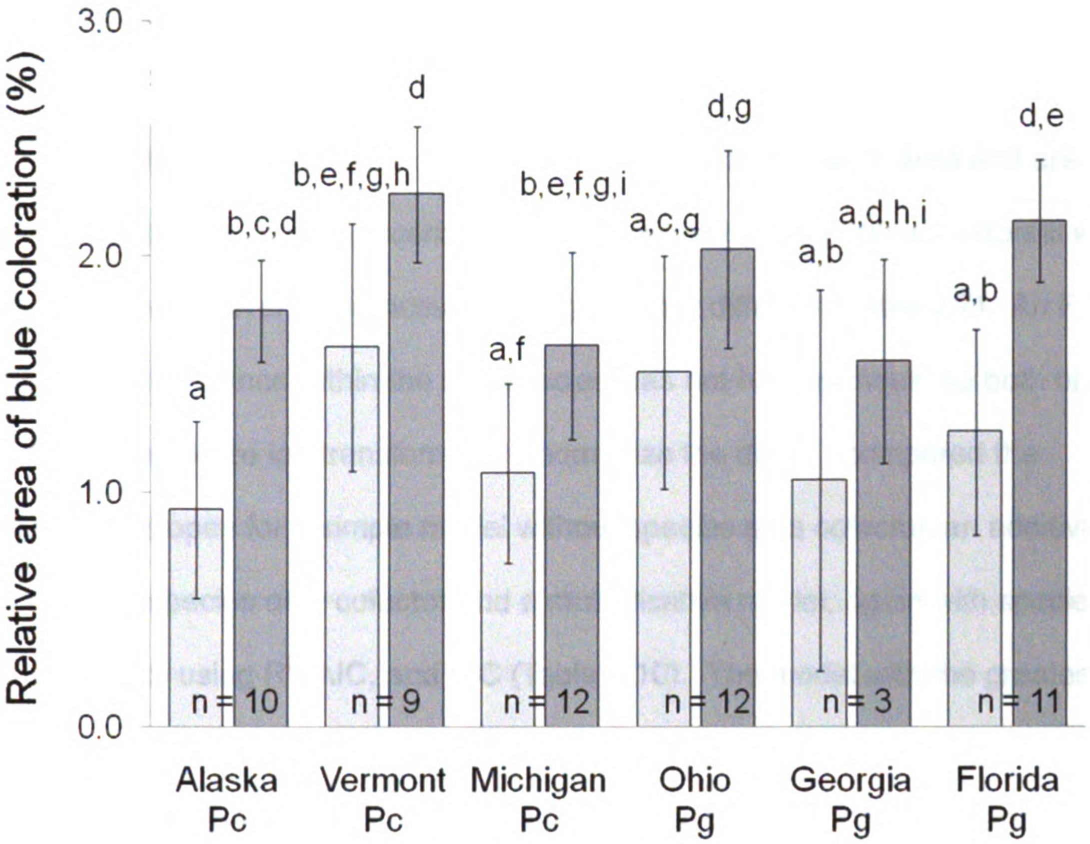

Figure 2.5. Mean relative blue area ± SD for all male *P. canadensis* (Pc) and *P. glaucus* (Pg). For each combination, the light grey bar shows the mean blue coloration on the dorsal wing surface and the dark grey bar shows the mean blue coloration on the ventral wing surface. Bars with the same letter are not significantly different at the p<0.05 level (pairwise t test with Holm's correction).

[F(4,49)=3.840, p=0.009]. Again, this cannot be generalized to species differences (Fig. 2.5).

I performed an analysis of covariance between total wing area and area of blue coloration for female *P. canadensis* and yellow-morph *P. glaucus* dorsal wing blue as species-level differences were significantly different (Table 2.7). An F test showed that variance within the two species was not homogenous, so both blue and total area were log transformed to normalize the data. I compared the regression slopes for a simple model without species as a cofactor, an additive model with species as a cofactor and a multiplicative model, again with species as a cofactor using R^2, AIC, and BIC (Table 2.10). The model with the greatest predictive value appears to be the additive model that incorporated population adjustments to the intercept. This suggests that within both female *P. glaucus* and *P. canadensis*, absolute blue area increases in a similar fashion (statistically equivalent slopes) from smaller to larger individuals, but that between females of the same size, *P. glaucus* females have fundamentally more absolute dorsal blue coloration (Fig. 2.6).

Ultraviolet photography of dorsal wing coloration revealed clear areas that reflect UV and areas that appear to be entirely UV absorbent (Fig 2.7). Reflectance appeared to occur primarily in the blue regions of the wing. The spectral analysis revealed a strong UV signature for blue coloration (Fig 2.8), but virtually no UV reflectance for yellow or black coloration.

Discussion

I examined differences in blue coloration between male and female tiger swallowtail butterflies and between the sister species *P. glaucus* and *P. canadensis*. As predicted, I found that males and females of *P. glaucus* differed significantly in the relative amount of blue on their dorsal wing surface, but not their ventral. However, I also predicted that female and male *P. canadensis* would differ significantly in their dorsal wing coloration, but this proved to be incorrect. Neither the dorsal nor the ventral amount of relative blue coloration differed significantly between the sexes in *P. canadensis.*

Females of each species differed from one another in their relative amount of dorsal blue coloration, but not their ventral. *P.* glaucus females had significantly more relative blue coloration than did *P.* canadensis females. Furthermore, regression analysis suggests that the area of blue coloration increases in a similar manner between *P. canadensis* and *P. glaucus*, but that *P. glaucus* has more blue on an absolute scale, irrespective of overall wing size. Unexpectedly, there were significant differences between male populations for both dorsal and ventral relative blue coloration, but these were not species specific. Finally, I found that blue coloration in general is UV reflective, whereas other coloration is not.

These observations lend support to my hypothesis that female-limited, dorsal coloration has importance for male mate recognition and possibly mate preferences. If males use the extent of blue coloration as a cue to differentiate between males and females than more blue coloration may result in a greater 'preference' for a particular female when multiple females are present. With

Table 2.1. Results from analysis of covariance (ANCOVA) for a comparison between female *P. canadensis* and yellow morph *P. glaucus* for the total area of the wings and the area of blue coloration with species as a cofactor. The data was $\log_{10}$ transformed to homogenize variance between the two species. The additive model appears to have the greatest predictive value and suggests that the two species increase in blue area in similar fashion with increased overall size (statistically equivalent slopes), but that *P. glaucus* females have more blue on an absolute scale. Bolded numbers represent the values for the preferred model in the three different analyses (Adj. R^2, AIC and BIC). See text for more details.

Model	**Species**	**Model Equation**	**Adj. R^2**	**AIC**	**BIC**
Simple	NA	$(\log_{10}\text{blue\_area}) = 2.104 * (\log_{10}\text{total\_area}) - 3.123$	0.768	-210.9	**-211.4**
Additive	*P. canadensis*	$(\log_{10}\text{blue\_area}) = 1.043 * (\log_{10}\text{total\_area}) - 1.951$	**0.836**	**-229.2**	-182.6
	P. glaucus	$(\log_{10}\text{blue\_area}) = 1.043 * (\log_{10}\text{total\_area}) - 1.636$			
Multiplicitive	*P. canadensis*	$(\log_{10}\text{blue\_area}) = 1.289 * (\log_{10}\text{total\_area}) - 2.231$	0.835	-228.0	-159.9
	P. glaucus	$(\log_{10}\text{blue\_area}) = 0.842 * (\log_{10}\text{total\_area}) - 1.362$			

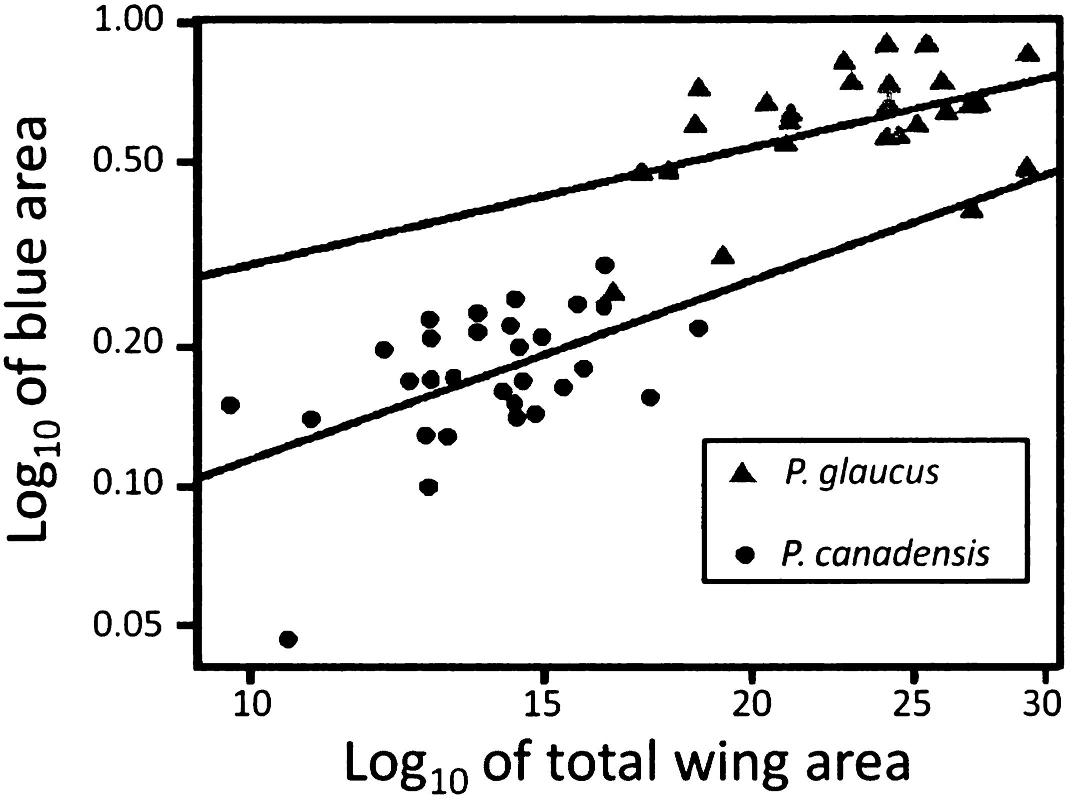

Figure 2.6. Log transformed comparison of absolute total wing area and area of blue coloration for *P. canadensis* and yellow morph, *P. glaucus* females. Area values are in cm^2. Regression lines are drawn using the additive model (See table 2.10). The slopes are not significantly different. See text for more details.

regard to previous interspecific preference comparisons (Deering and Scriber, 2002), my results suggest that more relative blue coloration may explain why males of both *P. glaucus* and *P. canadensis* preferred to mate with female *P. glaucus* over similar sized *P. canadensis* females. Although it is common to find multiple females feeding in close proximity to one another (Aardema, pers. obs.), most encounters likely involve single males and females. In these instances, the amount of dorsal blue may influence a male's initial inclinations to engage the female or the amount of effort given to pursuit if it occurs. Differences in blue coloration may also influence the amount of nutrients and sperm a male passes to the female in his spermatophore (Bonduriansky, 2001; Xu and Wang, 2009). It is not presently known whether male tiger swallowtails vary the size of their spermatophore based on a 'quality' evaluation of their mate. Although some unusual and extreme patterns in male blue coloration have been observed (Scriber, 1990b), more general variation in male blue coloration was unexpected. Perhaps even more surprisingly, this variation does not correspond to the two different species. It seems that deviation in blue coloration within the Michigan population explains most observations of difference. Michigan had a relatively small amount of blue both dorsally and ventrally compared to the majority of the other populations. It is presently unknown what factors might result in this population having less blue (or conversely, other populations having more blue).

Given the observed patterns of difference in blue coloration between the species and the sexes, mate recognition and/or preferences remain very likely candidates for selection pressures influencing the amount of blue on the wings,

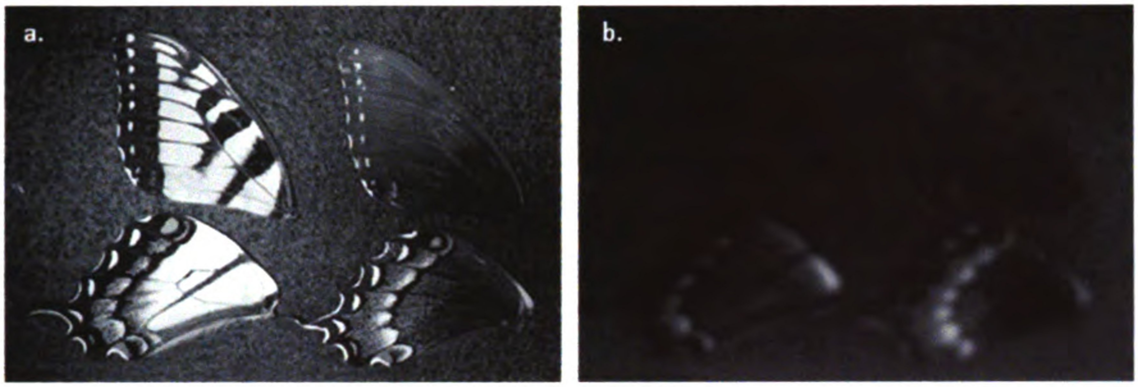

Figure 2.7. An example of my photographic examination of ultraviolet coloration in tiger swallowtail butterflies. a.) The visual image of the dorsal side of a yellow morph (left) and dark morph (right), female *P. glaucus*. b.) This image was taking using a UV bypass filter. The wings displayed are the same as those in 'a.' Areas that reflected UV light are white and areas that absorbed UV light are black. See text for more details.

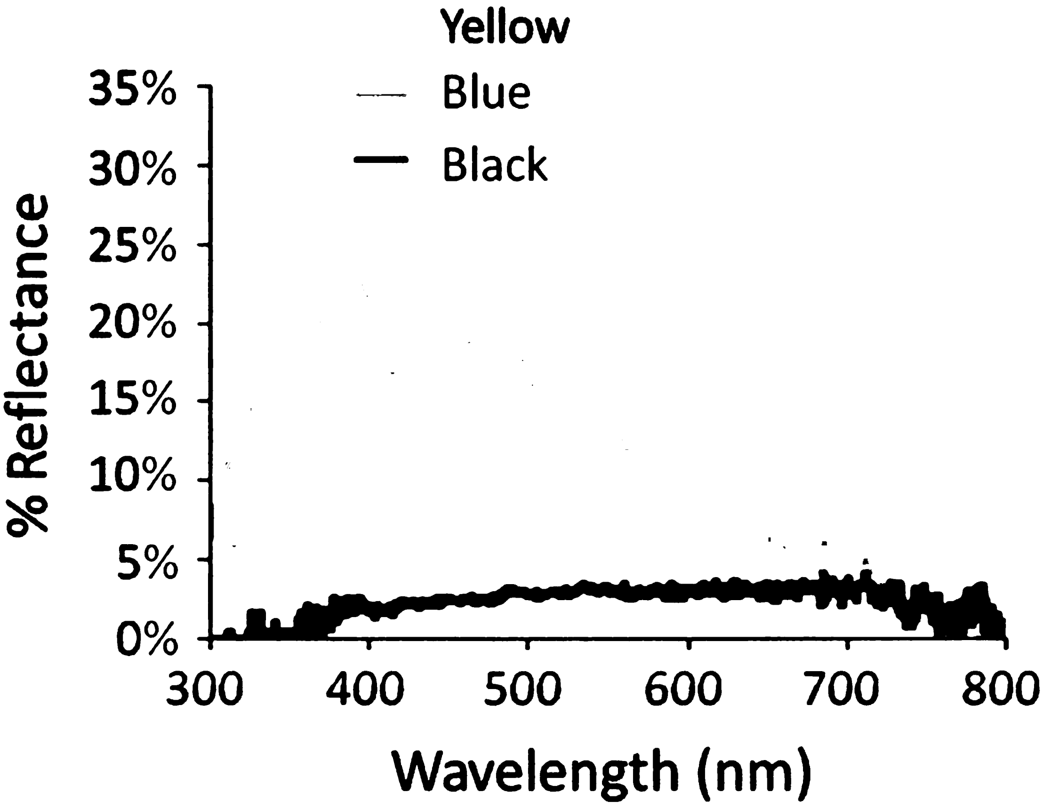

Figure 2.8. An example of the reflectance spectrum patterns of blue, yellow and black coloration from one yellow morph, *P. glaucus* female. The other three individuals assessed in this manner had similar spectral patterns. This figure shows a clear UV signature (300-400 nm) for blue coloration, and virtually no UV reflectance for yellow and black coloration. See text for more details.

especially for females. However, it is easy to speculate that amount of blue coloration may have multiple selection factors acting on it, some of which may be antagonistic to one another.

One pressure that may work to reduce blue coloration is avian predation. Birds have visual capabilities that extend into the UV spectrum and it has been shown that Lepidoptera with extensive ultraviolet coloration may be at a higher risk of attack relative to Lepidoptera that have little UV coloration (Church *et al.*, 1998; Majerus *et al.*, 2000, Lyytinen *et* al., 2004). It is presently unknown how predation pressure would act to reduce blue coloration in males and females, and given differences in daily behavioral activities (e.g. puddling in males versus ovipositing in females), it is possible that selection on the two sexes by avian predators differs.

Another influence on blue coloration may be temperature and a need for thermoregulation. Most ultraviolet coloration in lepidopterans is structurally based (Ghiradella, 1991; Prum *et al.*, 2006) and such structural color has been shown to significantly increase absorbance of solar radiation (Bosi *et al.*, 2008). However, two lines of evidence suggest this is unlikely to be a large selective force on blue coloration. First, the populations that would be predicted to have the greatest need for increased solar absorbance would be those located in more northerly localities. However, these populations have the least amount of blue coloration. Secondly, it is only the basal one-third of the wing that strongly influences thoracic and abdominal temperature (Douglas, 1981). This means that most blue coloration in both males and females is unlikely to strongly affect an individual's body temperature.

Yet another influence on the extent of blue coloration may be sexual harassment by other butterflies either of the same species or different species. Although polyandrous, female tiger swallowtails will in some circumstances refuse male mating attempts, likely based on whether they have recently mated and possibly due to perceived quality of the male. While females feed, they are often subjected to harassment by males (Lederhouse, 1995; Deering and Scriber, 2002; Aardema, pers. obs.). This type of intraspecific interaction has been shown to have negative fitness consequences for other insect species (Svensson *et* al., 2005; Takahashi and Watanabe, 2010). If males utilize blue as a mate recognition cue, than male harassment would likely oppose pressures acting to increase blue coloration due to a disproportionally greater amount of harassment directed towards females that have more extensive blue. Additionally, in some populations of tiger swallowtail, interspecific harassment occurs from males of other butterfly species (Deering and Scriber, 1998; Aardema, pers. obs.). Like intraspecific harassment, interspecific harassment likely has negative fitness consequences on females (McLain and Pratt, 1999; Nielsen and Watt, 2000). However, this pressure could act to either increase or decrease blue coloration depending on what recognition cues the males of other species react to. Furthermore, interspecific sexual harassment could have negative impacts on males in addition to females (Gröning and Hochkirch, 2008).

Although I have shown that dorsal blue coloration is significantly different between *P. glaucus* males and females, and between female *P. canadensis* and *P. glaucus*, its utility for mate recognition and/or mate selection is by no means a certainty. Only further examinations will elucidate whether blue coloration is actually utilized by males for the purpose of recognizing potential mates and/or judging these mate's reproductive quality. An investigation of these potential influences on tiger swallowtail blue, combined with studies of other selection factors, will help to reveal why variation in blue coloration exists and possibly what influences this variation could have on the dynamics of gene flow in the hybrid zone. Of most interest may be how pre-zygotic mating barriers are influenced by this seeming sexual characteristic, and how it contributes to divergence, reproductive isolation and adaptive radiation in the tiger swallowtail species complex.

CHAPTER 3:

COURTSHIP AND MATING RESPONSES OF MALE *PAPILIO* SPP. TO VARIATION IN FEMALE-LIMITED WING COLORATION

Introduction

Morphological traits that are limited to one sex and appear to have no direct utility for either basic survival or fostering offspring may be suspected to have some function in attracting and obtaining a mate (Darwin, 1879). These 'secondary sexual characteristics' may function either through intra-sexual conflict or intersexual mating preferences. General and consistent differences in these traits between closely related species may be further suspected of having importance in conspecific mate recognition and preference (Panhuis *et al.,* 2001), which may facilitate continued reproductive isolation.

I have shown that the relative amount of blue coloration on the dorsal side of the wings in female *P. glaucus* butterflies is significantly greater than in males (Chap. 2). Additionally, this blue coloration is relatively more extensive in female *P. glaucus* than in the sister species *P. canadensis*. Dorsal color between male and female *P. canadensis* does not differ significantly, nor does ventral blue color between males and females of either species. Lastly, females of the two species do not differ from one another in the relative amount of ventral coloration.

Males of both *P. canadensis* and *P. glaucus* prefer to mate with female *P. glaucus* in two-choice assays (Deering and Scriber, 2002). Given the observed interspecific variation in relative blue coloration, it stands to reason that this trait may be important for male mate recognition. Furthermore, although it is presently unknown how the extent of blue correlates with fitness, it is possible that this trait may be used in intraspecific mate selection. This would be especially true if there is a high degree of variation in the genetic quality of females (something presently unknown). In addition to the attributes of blue coloration listed above (interspecific and male/female variation), this morphological feature is UV reflective (Figs. 3.1&2) and found in both color morphs of female *P. glaucus.* It may thus act as a shared recognition characteristic between the color morphs that enables individual males to identify both colors as potential mates.

If the presence of blue coloration acts as an important mate recognition cue, and/or a potential mate selection cue, then I postulate that male tiger swallowtail butterflies of both *P. canadensis* and *P. glaucus* will strongly prefer to mate with females that exhibit a greater extent of blue coloration relative to females that exhibit less blue coloration. In an extreme case, I predict that when presented with females that either retain or lack dorsal blue coloration, males will predominantly choose females that have their blue preserved.

Materials and Methods

Butterflies

My studies were conducted during the summer from 2008 to 2010. For all years, I utilized adult, virgin females, captive reared from eggs laid by wild-caught mothers collected from Bennington Co. Vermont (VT; a *P. canadensis* population) and Lancaster Co. Pennsylvania (PA; a *P. glaucus* population). Although ideally I would have used females reared from the same location that the studies were conducted in, the large number of females needed made this impractical, and hence it was necessary to obtain females from sources that had the capacity to rear large numbers of individuals. As my comparisons were between females reared from the same populations, I feel that the origin of my butterflies did not influence the outcome of my results. This notwithstanding, in 2008 I was able to rear virgin females from wild mothers that I collected in Jackson Co. Ohio (OH; a *P. glaucus* population). For VT and PA females, I utilized individuals in the year following their rearing (i.e. they overwintered in diapause). The OH females were utilized in the same year in which I originally collected their mothers.

Experimental Regions

I conducted two-choice mate preference experiments in two *P. glaucus* populations, one during the first seasonal flight in Levy Co. Florida (in 2009 and 2010) and during both the first (2010) and second (2008) seasonal flights in Jackson Co. Ohio. For the Florida experiments I used all females reared from PA mothers. For the 2008 Ohio experiments (conducted during the second flight), I used females reared from mothers collected during the first flight of this population. In 2010, I used females reared from PA mothers. I also conducted two-choice experiments in a single *P. canadensis* population in Charlevoix Co., Michigan (in 2008). For this trial, I used all females reared from VT collected mothers.

All three regions were the same as those where females were collected for my blue coloration analysis (Chap. 2). In some cases, collection of females and the male mate-choice experiments occurred concurrently, but there is no reason to believe that this affected my results in either case.

Single choice experiments were conducted in 2009 in a *P. canadensis* population in Charlevoix Co., Michigan using VT *P. canadensis* and in 2010 in a *P. glaucus* population in Jackson Co, Ohio using PA *P. glaucus*. These were the same populations used in my two-choice studies. Based on the outcome of my two-choice assays and the single-choice experiments (see results), I deemed it unnecessary to conduct single-choice assays in Florida.

General Mate Choice Experimental Methods (Used in both 2- & single-choice assays)

To examine how male mating preferences were influenced by the extent of blue coloration on females, I conducted choice experiments in wild populations with known high concentrations of males. To do this, I manipulated females so that they either retained their dorsal blue coloration or had it entirely concealed. I choose to do these extremes, as it was my hope that this would result in the strongest differences and I could then look at more subtle variation in future work. In the areas described above, I presented these manipulated females to wild males. My methods are modified from Brower (1959) and Deering and Scriber (2002).

I first tied a fine nylon thread carefully around the thorax of fresh (emerged from the pupal stage within 10 days prior), virgin females between the front and hind wings. The other ends of these threads were tied to metal alligator clips which allowed me to attach the females to dowels that I inserted through holes in wooden stakes driven into the ground. My stakes were each approximately 2 meters long. The length of the thread varied between 40 and 60 cm. I attempted to have the females hang at a height similar to that of the nectaring plants that were being utilized by the study population. Typically, this was 1 – 1.5 m above the ground. To prevent excessive tangling, a small lead weight was attached to the thread halfway between the butterfly and the alligator clip. This prevented most tangling, although females would still occasionally fly on the thread in a manner which caused the line to kink. When this occurred, I manually untangled these females.

All females in this study received one of two treatments. For the experimental treatment (blue-concealed), I masked all dorsal blue coloration using ink from a fine tipped black sharpie brand marker. For the control treatment (blue-retained) I covered up black areas of the wing with this same black ink, but left all dorsal blue coloration visible. The area of coverage in both treatments was approximately equal. This black ink effectively concealed both the visual and UV spectral components of the blue coloration and appeared very similar spectrally to natural areas of black coloration (Fig. 3.1). I assigned females to one of these two treatments haphazardly without first looking at the extent of her dorsal blue coloration.

In most cases, I engaged in two tethering sessions each day, one in the morning and one in late afternoon. My previous observations indicated that these were the times when males would be most likely to initiate copulation. Tethering sessions typically lasted two hours. No female was ever left out for longer than three hours. In between tethering sessions, females were kept in a cooler with ice.

Two-choice Assays

Two-choice male mate preference trials consisted of preparing pairs of females in the manner described above. Within each pairing, the females were of approximately the same size as measured by forewing length from base to tip (± 1 mm). For the two-choice assay the wooden dowel was placed through the top of the stake so that equal halves stuck out horizontally in opposite directions. This allowed me to tether females approximately one meter apart. The stakes were placed a minimum of eight meters from one another either along roads that were being used as flyways (Ohio and Florida) or else scattered about a meadow where there were abundant nectar resources (Michigan).

Males flying by these females would engage them if interested. Typically this involved repeatedly circling of the female on her tether. Upon contact with a male the female would generally become enlivened and flutter rapidly at the end of the string, spiraling in tight circles. At this point the male would either land on the female and initiate copulation or in some cases fly off. In many instances the male would engage both females of a pair before copulation was initiated.

While tethered, I checked each pair at least once every five minutes. After I observed a mating being initiated or else found a copulation in progress, I would gently remove the male from the female. In most cases, I retained males for future analysis as part of a separate study. Otherwise, I released males back into

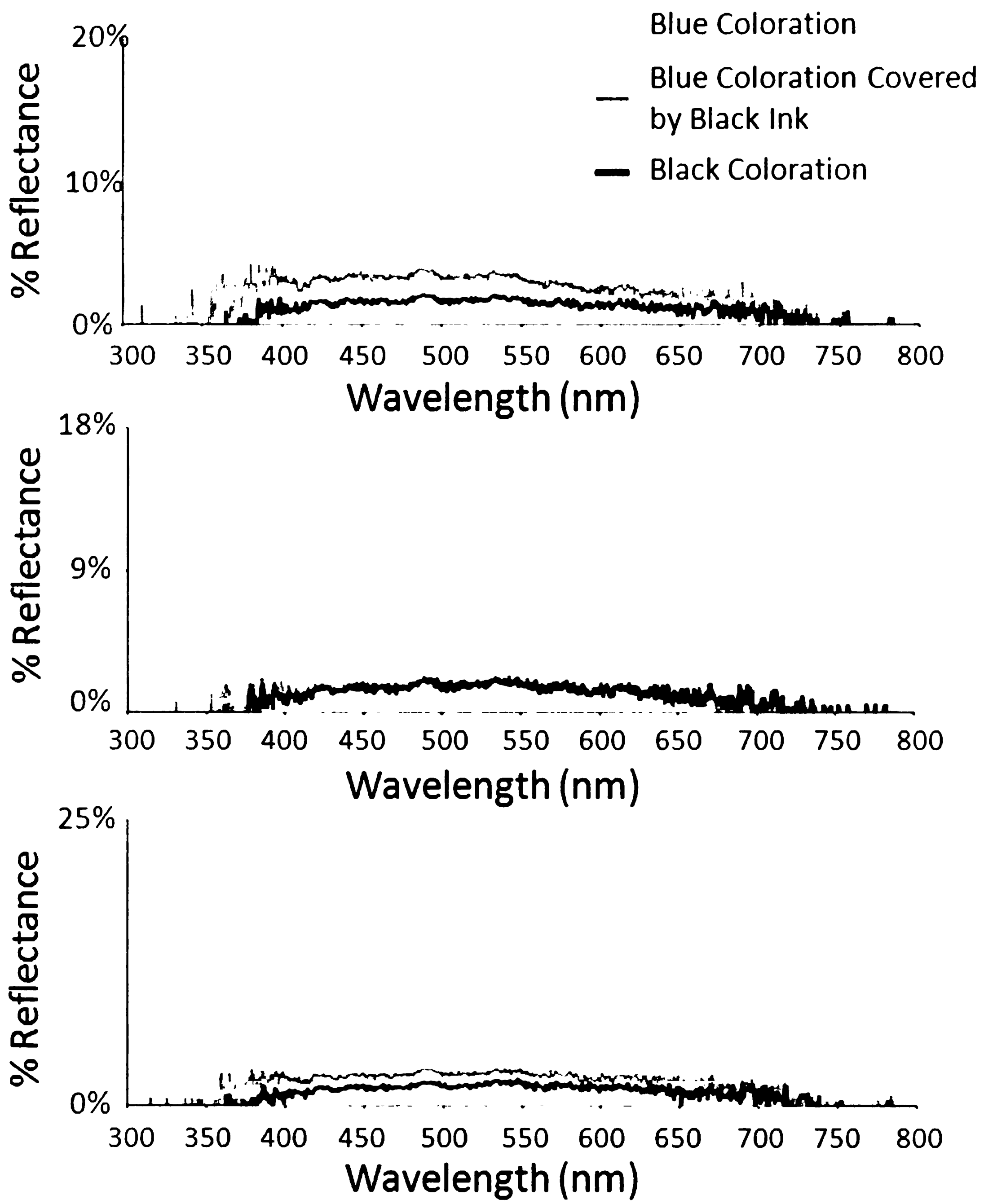

Figure 3.1. Three examples of female *P. glaucus* dorsal colors showing the spectral reflectance signature of blue coloration, black coloration and blue coloration covered by black ink from a sharpie brand marker. UV coloration is that which occurs between 300 and 400 nm. Black ink effectively conceals both the UV and visual elements of the blue coloration.

the population after they had been marked with a unique number using a red sharpie and the 1,2,4,7 numbering system developed by Ehrlich and Davidson *(*1960*)*. Most marked males were not seen again, but in a few cases the marked males would return and attempt to mate with a different female in another pair or occasionally the same female. I did not include any pairing with the same male and female twice. I did, however, include multiple mate choices by the same male for different females. These second choices account for four of my total number of pairings. After each copulation, I switched the positions of the two females on the tethering pole. There were no more than three total copulations with any collective pair during a single tethering session.

Single-choice Assays

It has been suggested that choice experiments such as my two-choice assay may lead to erroneous conclusions as an element of one of the females may elicit courtship behavior in males, and after this occurs no further preference is exhibited (Tinbergen, 1948). It is possible that the female that retained her blue coloration compelled males to engage in mating behaviors and the female he inevitable ended up with was not indicative of a choice. To account for this possibility, I also carried out single-choice trials where treated females would be available to males without a female exhibiting the opposite treatment in close proximity.

For the single-choice assay, I tethered females singularly to dowels in a fashion similar to that used in the two-choice assay except that only one female was present rather than two. At all times during the trials there were equal numbers of blue-concealed and blue-retained females available in the study area. These females were not sized matched between the treatments. The total number of individuals I tethered out during a given session varied from 6 to 14, depending on availability. I kept single females at a minimum distance of eight meters apart. Treated females were assigned to poles haphazardly and when a male initiated copulation with a female, I exchanged her for a female of the opposite treatment.

Statistical Analysis

I performed all analysis with the statistical package R (Abramoff *et* al., 2004). To assess whether the number of males that preferred females with blue retained was significantly greater than the number of males which preferred females with blue concealed, I used chi-square tests to compare the number of matings for each treatment to the expected null-distribution of 0.5, which would indicate random mating. I assessed each population in this manner individually, with dark and yellow morphs separate and combined. Additionally, I combined all observations for each treatment together. When the same experiment was conducted in multiple years, I combined observations for my analysis. I did not analyze years separately as samples sizes were generally small. Finally, in cases where my sample sizes were too low (expected values <5.0), I did not assess the data statistically.

Results

Two-Choice Trials

When all two-choice pairings were totaled, there were 34 male copulations with females of both species and color morphs that had their blue-retained, and 44 copulations with blue-concealed (Table 3.1). This overall result is not significant, $\chi^2(1, N = 78) = 0.26, p = 0.61$.

In the Florida population (*P. glaucus*), I observed 5 copulations with blue-retained, yellow-morph females, and 7 pairings with blue-concealed, yellow-morph females. This difference was not significant, $\chi^2(1, N = 12) = 0.56, p = 0.45$. For dark-morph females, I observed 5 pairings with blue-retained and 8 with blue-reduced. This difference was also not significant, $\chi^2(1, N = 13) = 0.41, p = 0.52$. When I compare all the pairings of both color-morphs (10 blue-retained, 15 blue-concealed), the result was not significant, $\chi^2(1, N = 25) = 0.32, p = 0.57$.

Within the Ohio population (*P. glaucus*), there were 6 mating observations with yellow-morph females with blue-retained and 5 with blue-reduced. This difference was not significant, $\chi^2(1, N = 11) = 0.76, p = 0.38$. For dark-morph females I observed 7 pairings for blue-retained, and 8 for blue-reduced. Again, this difference was not significant, $\chi^2(1, N = 15) = 0.80, p = 0.37$. A statistical comparison of all the pairings of both color-morphs (13 blue-retained, 13 blue-concealed) was unnecessary as the number of pairings for each treatment was identical.

In the Michigan population (*P. canadensis*), I obtained 11 pairings with females that retained their blue and 16 pairings with females that had their blue

Table 3.1. Number of two-choice pairings for each population examined. When male preferences were examined over multiple years for the same population, results are combined. No difference was significant (see text for values)

Population	**Species**	**Female Color Morph (*P. glaucus* only)**	**Total Number of Pairings**	**Number of Pairings with Blue-Retained Female**	**Number of Pairings with Blue-Concealed Female**
Florida	*P. glaucus*	Yellow	12	5	7
		Dark	13	5	8
Ohio	*P. glaucus*	Yellow	11	6	5
		Dark	15	7	8
Michigan	*P. canadensis*	NA	27	11	16
TOTAL	**BOTH**	**ALL**	**78**	**34**	**44**

concealed. This was not a significant difference, $\chi^2(1, N = 27) = 0.34$, $p = 0.56$. Within the two *P. glaucus* populations, I compared the observations for all dark-morph and yellow-morph females. For the yellow, there were 11 observations of pairing with blue-retained females, and 12 observations with blue-reduced females. This was not a significant difference, $\chi^2(1, N = 23) = 0.83$, $p = 0.36$. I obtained 12 total pairings with blue-retained, dark-morph females, and 16 pairings with blue-reduced, dark-morph females. Again, this was not a significant result, $\chi^2(1, N = 28) = 0.45$, $p = 0.50$.

One-Choice Trials

Combining all one-choice pairing observations from both Michigan and Ohio, I observed 16 pairings with females that had their blue retained and 21 pairings with females that had their blue reduced (Table 3.2). This difference was not significant, $\chi^2(1, N = 37) = 0.41$, $p = 0.52$. My Ohio samples for both yellow and dark morph females were too small to compare using the chi-square test, but for yellow-morphs, there were 2 pairings with blue-retained females, and 2 pairings with blue-reduced females. For dark-morph females, there were again 2 pairings with blue-retained, and 3 with blue reduced. Examining just the Michigan population, I observed 11 pairings with females that retained their blue and 17 pairings with females that had their blue reduced. This result was not significant, $\chi^2(1, N = 28) = 0.26$, $p = 0.61$.

Table 3.2. Number of one-choice pairings for each population examined. When male preferences were examined over multiple years for the same population, results are combined. No difference was significant (see text for values).

Population	**Species**	**Color Morph (*P. glaucus* only)**	**Total Number of Pairings**	**Number of Pairings with Blue-Retained Female**	**Number of Pairings with Blue-Concealed Female**
Ohio	*P. glaucus*	Yellow	4	2	2
		Dark	5	3	2
Michigan	*P. canadensis*	NA	28	11	17
TOTAL	**BOTH**	**ALL**	**37**	**16**	**21**

Discussion

I examined male mate recognition and preference for females with either their dorsal blue coloration retained or concealed in one- and two-choice trails. My results suggest that an absence or reduction of dorsal blue coloration on a female does not reduce a male's willingness to engage in copulation with her. Males readily mated with females that had their entire dorsal blue coloration concealed. This result was surprising as several lines of indirect evidence suggest that blue coloration may be important for male mate recognition and possibly mate preference.

Although not significant, there may actually have been a slight trend for males to prefer females that had their blue concealed. In all but two comparisons (both in Ohio, 'all pairings' and 'yellow-morph, *P. glaucus* females'), the number of pairings was always greater for females with blue-concealed than for females with blue-retained. I attribute this to my low sample sizes and believe that an increase in the number of pairings would eliminate this slight trend. Nonetheless, it is possible that a significant preference for blue-concealed females would be seen with additional pairings. However, given the original secondary evidence that initially lead to this investigation, it is unlikely that this would be the case as this would predict selection pressure for a reduction in the extent of blue coloration. The continued presence of a large amount of female-limited blue would, in turn, suggest a strong counter agent selecting for the retention of blue coloration. Given my results, no such agent is presently known.

One problem with focusing on a single trait (such as blue coloration) is that in most systems male mate recognition and preferences are likely to be based on several concurrent and possibly interacting traits (Bonduriansky, 2001). The most important factor influencing insect male mate choice is generally the fecundity of the female or the number of eggs she has available. Genetic quality is almost certainly second in terms of selection importance (Bonduriansky, 2001). If blue were an important characteristic used in mate preference, than it would likely advertise this second component of fitness, and thus be less important than indicators of fecundity such as body size. In the context of my experiments, the absence of blue coloration (if it had any influence), may have been overshadowed by the unmated state of my treated females and their correspondingly large abdomens.

Likewise, pheromonal cues could also have played a role in male mate selection. Olfactory cues have an important role in a vast number of Lepidopteran systems where male mate selection is observed (Bonduriansky, 2001), and may be the most common form of mate assessment. Although previous pheromonal analysis conducted within the tiger swallowtail system suggests that pheromones have limited influence on males (Deering, 1998), this research did not rule out close-range or contact olfactory influences on mate selection.

In general, sexual characteristics which would advertise fecundity are likely to be redundant and the resources spent on them better applied to increasing the number or success of offspring (Fitzpatrick *et al.*, 1995). This notwithstanding, when the number of matings a male can achieve is limited (independent of access to mates) such as is the case with tiger swallowtails, than female genetic quality can significantly impact a male's lifetime fitness (Bonduriansky, 2001). For instance, individuals may differ in their ability to utilize locally common hosts, and this may be reflected in morphological characteristics. If a male can recognize this, than it becomes beneficial to mate with females that exhibit traits which indicate they use hosts well.

Most likely, a combination of visual, olfactory, and tactile cues combine in some sequence to create an overall impression of a female to males (Bonduriansky, 2001). If one of these elements is removed, the high favorability of others may be enough to overcome this deficiency. In the end, the presence of such multiple traits may have lead to an apparent lack of importance for blue coloration in this system.

Male 'choice' may also be expressed cryptically as variation in the amount of sperm and/or nutrients a male passes on to a particular female (Bonduriansky, 2001). It is possible that male tiger swallowtails may have exhibited this type of mate choice in response to variation in the presence of blue coloration.

Rather than classification as a secondary-sexual characteristic, female limited blue coloration may be better explained in the context of the *Battus philenor* mimicry complex, of which dark-morph females are considered a part of (Brower, 1958). *B. philenor* males have extensive blue coloration on their dorsal hind wings, and are a model for several mimetic species. Mimicking this coloration likely provides some level of protection from predators, and accordingly many edible species whose range is congruent have very similar coloration (Brower and Brower, 1962; Mullen *et al.*, 2008). The underlying genetics which result in differential dorsal blue coloration are unknown although it would seem that the genetic regions that contribute to the generation of extensive female blue coloration are separate from the regions that confer dark or yellow morph coloration respectively, as both morphs clearly have extensive blue independent of their base coloration. It is possible that there is strong selection on dark-morph females to have large amounts of blue coloration and that this carries over into yellow-morph females. Likewise, this trait may also either be retained in *P. canadensis* populations from its progenitor or else maintained by contemporary or historically recent introgression. Evidence from microsatellites and other traits that do not have strong divergent selection on them indicate that gene flow on such regions across the hybrid zone is high (Nowak *et al.*, 2009). Selection for a large amount of blue coloration in dark-morph females may hence carry over into both yellow morph females and also into *P. canadensis* populations.

This alternative hypothesis is not without problems. The blue coloration in tiger swallowtails is strongly UV reflective whereas the blue coloration of *B. philenor* is UV absorbent (Figure 3.2). Most birds have visual acuity which extends well into the ultraviolet range (Chen *et al.*, 1984). This suggests that differences in UV reflectance between *B. philenor* and dark-morph, female *P. glaucus* may be distinguishable to birds and reduce the effectiveness of this mimicry coloration. Furthermore, ultraviolet coloration in diurnal lepidoptera has been shown to increase predation risk (Cuthill and Bennett, 1993; Majerus *et* al., 2000; Lyytinen *et al.*, 2004). Hence this UV coloration may make yellow-morph females more conspicuous to predators relative to the blue-reduced coloration of males.

It is clear that additional experimentation is needed to determine both why female tiger swallowtails retain a greater amount of dorsal blue coloration than males, as well as what traits males use in mate selection (other than blue) that resulted in the asymmetrical male matings preferences for *P. glaucus* females.

CHAPTER 4:

POSSIBLE ALTERNATIVE EXPLANATIONS FOR ASYMMETRICAL MALE MATING PREFERENCES IN *PAPILIO* SPP.

Introduction

In tiger swallowtail butterflies, a large amount of secondary evidence suggests that dorsal blue coloration is an important cue for mate recognition and possibly mate selection. This evidence includes three observations: 1) dorsal blue coloration is significantly most extensive in female *P. glaucus* relative to both male *P. glaucus* and all *P. canadensis* (see chapter 2), 2) blue is likely highly visible to these butterflies due to substantial ultraviolet reflectance (Briscoe, 2000; chapter 2) and 3) this blue coloration may be a shared characteristic between the otherwise visually very divergent dark and yellow morph females that occur in some *P. glaucus* populations (see chapter 2). Combined with repeated demonstrations that males exhibit mate preferences (Bower, 1959; Deering and Scriber, 2002, Aardema and Scriber, unpublished data), this evidence suggests that blue coloration may be important in mate recognition and possibly mate choice in these butterflies.

However, as I have shown (Chapter 3), blue coloration in females is unlikely to have a significant on male mate choice in these tiger swallowtail populations. Experimental alteration of this trait did not elicit any differential mating response from wild male populations of *P. canadensis* in Michigan or *P. glaucus* in Ohio and Florida.

Two questions arise from this outcome. First, if blue coloration is not important in mating preferences, does it have other biological significance? I addressed this question in chapter three, postulating that the one explanation for patterns of variation in blue coloration may be its utility to dark morph *P. glaucus* females for mimicking the toxic, pipevine swallowtail, *Battus philenor*. However, this use may be conflicted by the UV component of the blue scaling and the affect this has on avian predation frequencies. Needless to say, uncertainties remain.

The second question concerns the traits males *are* using to choose mates. Put another way, what else varies between *P. canadensis* and *P. glaucus* females that may explain why *P. canadensis* males strongly prefer *P. glaucus* females? (Deering and Scriber, 2002). The list of possible traits may be long, but the most likely candidates are differences in abdomen size and variation in yellow coloration. Larger abdomens are a very common trait used by males to select females in other insect systems due to their direct correlation with fecundity (Bonduriansky, 2001). Female yellow variation is an important part of mate recognition and preferences in other butterfly systems (Lederhouse and Scriber, 1996; Ellers and Boggs, 2003), and would likely be a very conspicuous cue if utilized by males for the purpose of recognizing and selecting a potential mate.

I postulate that *P. glaucus* females have a fatter (heavier) abdomen compared to *P. canadensis* females relative to overall size as indicated by wing length. With regards to yellow coloration, it is hard to conjecture what characteristics male *Papilio* spp. prefer, but I hypothesized that some measureable value of yellow coloration will significantly differ between *P. glaucus* and *P. canadensis* females, and that this difference may have influence on male preferences.

Materials and Methods

Abdomen Size

To investigate differences in abdomen size I made use of 24 captive-reared Vermont (Bennington Co.) *P. canadensis* and 24 captive reared Pennsylvania (Lancaster Co., n=21) or Georgia (Oglethorphe Co., n=3) *P. glaucus* females. These butterflies were all initially reared in 2009. I kept them in diapause in 4°C over winter (2009/2010) for approximately eight months and then placed them in 23°C to emerge as adults. After emergence, these butterflies were kept refrigerated until I measured and weighed their abdomens.

I first measured the length of the forewings to the nearest 1 mm from base to tip with a standard metric ruler. This is a common approximate measurement for butterfly size (see chapter 1 for more details). Next, I removed the head followed by the abdomen at the 'waist' where it connects to the thorax. After removal the abdomen was quickly weighed to the nearest 0.001 gram.

Yellow Coloration

I examined yellow coloration by measuring the spectral reflectance signature of yellow dorsal areas using an Ocean Optics s2000 miniature fiber optic spectrophotometer. I took spectral reflectance measurements from 2 -3 separate yellow areas of the forewing from 17 *P. canadensis* females and 17 *P.*

glaucus females. The minimum area for spectral measurements is approximately 0.75 cm, and hence the areas selected for measurement came from wing regions that had a solid yellow patch at least this size without any other interfering colors. Females were collected from Fairbanks-North Star Co., Alaska (n=9, *P. canadensis*), Charlevoix Co., Michigan (n=8, *P. canadensis*), Jackson and Gallia Cos., Ohio (n=6, *P. glaucus*) and Levy Co., Florida (n=11, *P. glaucus*). Reflectance of color was measured in comparison to a white standard. Using the reflectance values, I was able to calculate hue and chroma for each observation. Hue is essentially the color we recognize and consider as distinctive (e.g. "yellow", "blue", etc.), and chroma is a measure of the saturation or amount of diffusion of this color. These values were calculated based on Endler (1990), with modifications from Sobel *et al.*, (In Prep.). The mean of multiple measurements from single individuals was used in species and population comparisons.

Statistical Analysis

Abdomen size (as determined by weight), was divided by wing length to get a relative measurement of the relationship between the two values for *P. canadensis* and *P. glaucus* females. Using these measurements, I compared the variances using an F test (to determine homogeneity) and then Students t-test to determine it the means were statistically different for the two species.

I also analyzed the covariance between wing length and abdomen weight using species as a cofactor. I compared three models: a simple model that did not take species into account, an additive model with species as a cofactor and a multiplicative model, again with species as a cofactor. Models were compared using adjusted coefficient of determination (Adj. R^2), the Akaike information criterion (AIC; Akaike, 1978) and the Schwarz Bayesian Information Criterion (BIC; Schwarz, 1978).

Yellow coloration was compared for both hue and chroma between the two species using either Student's t test when variances were statistically equal or the Welch two sample t test when variances were unequal. Variance was examined using an F test. Hue and chroma were also compared between populations using an analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) for pairwise comparisons. For all analysis an alpha value < 0.05 was considered significant

Results

Abdomen Size

Summary statistics for the relative comparison between abdomen weight and wing length are displayed in table 4.1. Variances between *P. canadensis* and *P. glaucus* were homogeneous. There was a statistically significant difference between these two species [$T(46)=-4.433, p<0.001$].

In contrast to the relative abdomen size comparison described above, my analysis of covariance revealed that there was not a significant effect of population for my regression models (table 4.2, fig. 4.1). The slopes and y-intercepts are statistically equivalent, and the simple model which does not take

Table 4.1. Summary statistics for the relative relationship between forewing length (mm) and abdomen weight (g) in female *P. glaucus* and *P. canadensis*.

Species	**Observations**	**Mean**	**Standard Deviation**
P. canadensis	24	0.450	0.115
P. glaucus	24	0.628	0.159

Table 4.2. Results from analysis of covariance (ANCOVA) for a comparison between *P. canadensis* and *P. glaucus* females for wing length and abdomen weight with species as a cofactor. The simple model which does not take into account species appears to have the greatest predictive value and suggests that the two butterflies do not differ significantly in their basic allometric relationship between wing length and abdomen weight. Bolded numbers represent the values for the preferred model in the three different analyses (Adj. R^2, AIC and BIC). See text for more details.

Model	**Species**	**Model Equation**	**Adj. R^2**	**AIC**	**BIC**
Simple	NA	weight = 0.016 * wing_length - 0.536	**0.597**	**-263.01**	**-119.17**
Additive	*P. canadensis*	weight = 0.015 * wing_length - 0.469	0.591	-261.39	-115.69
	P. glaucus	weight = 0.015 * wing_length - 0.451			
Multiplicative	*P. canadensis*	weight = 0.012 * wing_length - 0.337	0.588	-260.12	-112.55
	P. glaucus	weight = 0.017 * wing_length - 0.580			

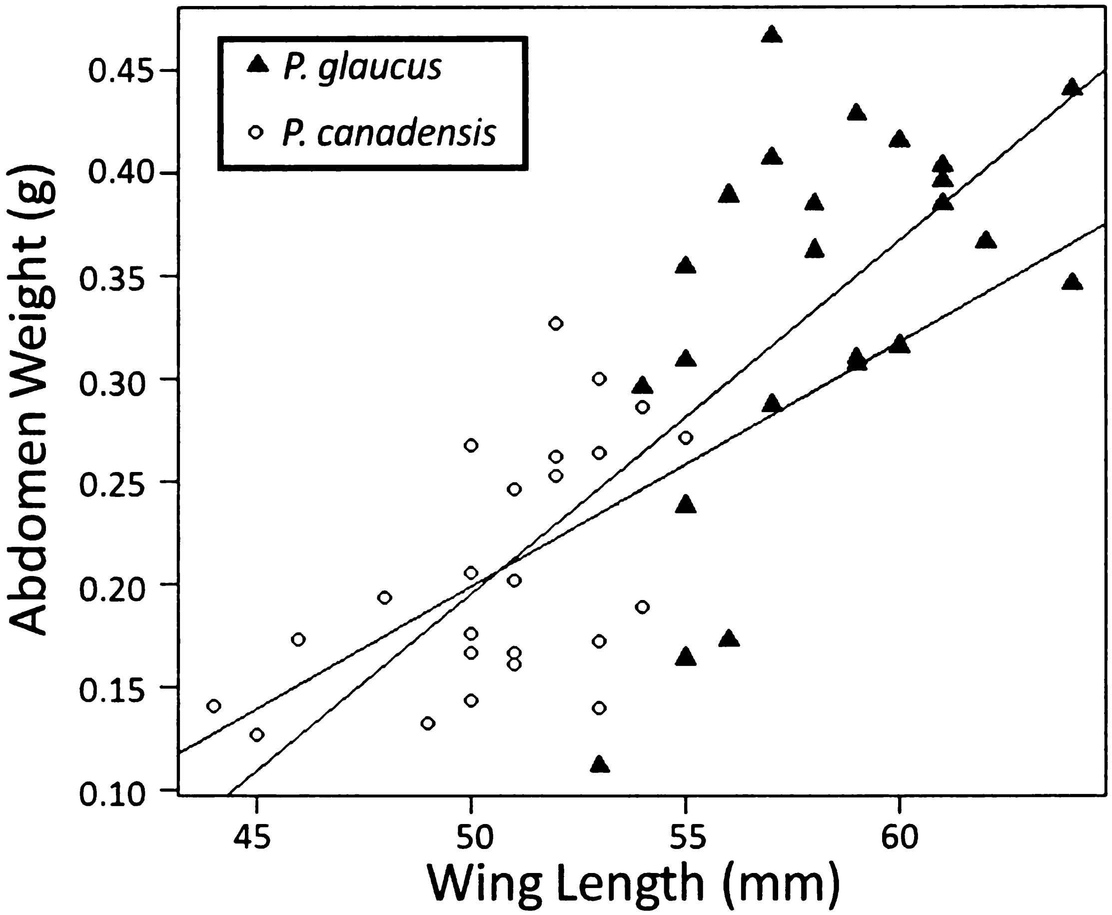

Figure 4.1. Comparison of wing length (mm) and abdominal weight (g) in *P. canadensis* and yellow morph, *P. glaucus* females. Regression lines are drawn using the simple model (See table 4.2). The slopes are not significantly different. See text for more details.

species into account was determined to be the best based on adjusted R^2, AIC and BIC values.

Yellow Coloration

Summary statistics for species' hue and chroma comparisons are presented in tables 4.3 a&b, and for population comparisons in tables 4.4 a&b. The variance between *P. glaucus* and *P. canadensis* for hue was homogeneous and the difference was significant [T(32)=4.463,p<0.001]. For chroma however, the variances were not homogonous, and the means were not significantly different [T(19.654)=-1.217,p=0.238].

In line with the results found in my species analysis, analysis of variance showed that there was a significant difference between populations for hue [F(3,30)=9.998,p<0.001], but not for chroma [F(3,30)=0.998,p=0.407]. For hue, observed significance was the result of differences between the Florida *P. glaucus* samples and samples from the two *P. canadensis* populations (Alaska and Michigan). The *P. canadensis* samples were not significantly different from the Ohio *P. glaucus* samples (P values from Tukey's HSD are presented in table 4.5).

Discussion

I examined two possible morphological differences between *P. canadensis* and *P. glaucus* that may explain asymmetrical male preferences for *P. glaucus* females relative to *P. canadensis* females. These were abdomen weight (as a proxy for 'size') and yellow coloration. I found that *P. glaucus* females have

Table 4.3a. Summary statistics for hue value in female *P. glaucus* and *P. canadensis*

Species	Observations	Mean	Standard Deviation
P. canadensis	17	0.245	0.022
P. glaucus	17	0.265	0.066

Table 4.3b. Summary statistics for chroma value in female *P. glaucus* and *P. canadensis*

Species	Observations	Mean	Standard Deviation
P. canadensis	17	1.276	0.082
P. glaucus	17	1.123	0.115

Table 4.4a. Summary statistics for hue value in populations of female *P. canadensis* (Alaska and Michigan) and *P. glaucus* (Ohio and Florida)

Population	Observations	Mean	Standard Deviation
Alaska	9	1.266	0.084
Michigan	8	1.287	0.085
Ohio	6	1.202	0.109
Florida	11	1.08	0.096

Table 4.4b. Summary statistics for chroma value in populations of female *P. canadensis* (Alaska and Michigan) and *P. glaucus* (Ohio and Florida).

Population	Observations	Mean	Standard Deviation
Alaska	9	0.246	0.026
Michigan	8	0.243	0.019
Ohio	6	0.245	0.028
Florida	11	0.276	0.078

Table 4.5. Significance (p) values from Tukey's HSD for all pairwise comparisons between populations in the yellow color analysis between *P. canadensis* (Alaska and Michigan), and *P. glaucus* (Ohio and Florida). Values on the lower left side of the figure represent chroma comparisons and values on the upper right side of the figure represent hue comparisons. Bolded values in the dark gray boxes are significant ($p \leq 0.05$). See text for more details.

	Alaska	**Michigan**	**Ohio**	**Florida**
Alaska		0.965	0.571	**<0.001**
Michigan	0.999		0.346	**<0.001**
Ohio	1.000	1.000		0.065
Florida	0.545	0.483	0.613	

Hue

Chroma

relatively heavier abdomens (in relation to wing length), but an absolute comparison of the two traits revealed a similar allometric relationship between wing length and abdomen weight for both species. This suggests that similar sized individuals (as indicated by wing length) should have similar abdomen sizes regardless of species. Why then the relative differences were significant is unclear. One explanation may be that the observations for each species were not spread out evenly across the complete distribution, but rather most of the *P. glaucus* observations occurred at longer wing lengths and heavier abdomens weights, whereas most of the *P. canadensis* observations were for shorter wing lengths and correspondingly lighter abdomen weights.

For yellow, the mean hue value was significantly different between *P. canadensis* and *P. glaucus* females, but not chroma. This was mostly due to Florida *P. glaucus* observations deviating from the other three populations. This suggests that the fundamental 'color' is different between the two species, or at least between Florida and the other populations. Color has immense importance for many other components of fitness (e.g. thermoregulation; Ellers and Boggs, 2003), and how males respond to females of different colors is unknown at this time.

To that end, both of these examinations are extremely preliminary and should be considered mostly in the context of future areas for study. Nonetheless, in both cases results appear promising for potential differences between the two species that may help to explain male mating preferences. Of the two, abdomen size is arguably the more likely candidate for a mate preference cue, even if it is not strongly different between the two species. A large abdomen likely would suggest more eggs and correspondingly more potential offspring to a male, or else larger eggs that may result in offspring more likely to survive the early stages of their life (Fischer *et al.* 2002). It may be possible that a female with a smaller abdomen would be less pursued by males or even rejected outright. Careful study of how these two traits vary between *Papilio* species and populations as well as how they influence male mate preferences will likely reveal a great deal of useful information regarding both the tiger swallowtail butterflies specifically and the evolution of mating systems in general.

CHAPTER 5:

SUMMARY AND CONCLUSIONS

The divergence of tiger swallowtail butterflies is a fascinating example of how many evolutionary influences can interact to generate new species. Vicariance, sexual selection, adaptation to divergent climates, host use divergence, mimicry and hybridization have likely all played important roles in the diversification of this small group.

I examined one component of sexual selection in tiger swallowtails in the hope of better understanding the role of male mate recognition and preferences in their diversification. Specifically, I examined variation in coloration between two common species, the Canadian tiger swallowtail, *Papilio canadensis* and the eastern tiger swallowtail, *P, glaucus*.

I found that the amount of dorsal blue coloration in females of *P. glaucus* was significantly more extensive then this same coloration on male *P. glaucus* and both male and female *P. canadensis*. I also found that this coloration was strongly UV reflective; making it likely that it is highly visible to other tiger swallowtail individuals.

Previous research found that males of both species preferred to mate with *P. glaucus* females when given the choice between these and similarly sized *P. canadensis* females. This observation, combined with my finding that *P. glaucus* females have a large amount of blue coloration, suggested that this blue was a good candidate for a trait males may use to distinguish and select females. I investigated this possibility using one- and two-choice mate preference experiments and found to my surprise that males do not appear to select mates based on the extent of their dorsal blue coloration

Perhaps the most important lesson that can be drawn from this work is the necessity of conducting rigorous experimentation before assigning utility to a morphological characteristic. Certainly, in most cases the hypothesized function of a trait will be found to be correct, but there may also be other, ancillary functions or, as in the case of blue coloration on tiger swallowtail females, the postulated purpose will turn out to be completely incorrect.

Another interesting finding of this work is in the illumination of areas where there is clear morphological divergence between *P. canadensis* and *P. glaucus*. Although it was qualitatively recognized that blue coloration appeared to be more extensive in *P. glaucus* females, this observation has now been quantified and verified. Additionally, it was previously unknown how these butterflies appeared in the ultraviolet spectrum, something which has now been assessed.

Although I ultimately disproved the importance of dorsal blue coloration in male mate selection, I have opened up many paths for future research on the influence of blue in other areas such as mimicry, thermoregulation and sexual conflict with conspecifics or other species. It may be particularly illuminating to investigate the apparent inconsistency between the visual blue coloration and the UV coloration of dark morph *P. glaucus* females. Furthermore, the problem of what traits males were cuing in on that generated overwhelming preference for *P. glaucus* females remains. I have shown that variation in yellow coloration and abdomen size may be good candidates to explain this, but both remain to be tested.

Overall, tiger swallowtails compose a diverse, complicated and fascinating clade that have the potential to maintain scientific inquiry for many years to come. There is little doubt that this inquiry will lead to a better understanding of how variation in organic beings influences and is influenced by evolutionary processes. Likewise, it will generate much general information that will contribute to our knowledge of the natural world.

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.: 2010-06

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

THE INFLUENCE OF FEMALE WING COLOR VARIATION ON MALE MATE CHOICE IN TIGER SWALLOWTAIL BUTTERFLIES (*PAPILIO* SPP.)

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Matthew L. Aardema

Date Aug. 3, 2010

*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

Page_1__of_1__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 glaucus	FLORIDA Levy Co.					3			MSU
Eastern Tiger Swallowtail	Cedar Key Scrub State Reserve								
	4-Apr-2006 J.M. Scriber								
Papilio glaucus	FLORIDA Levy Co.						3		MSU
Eastern Tiger Swallowtail	Cedar Key Scrub State Reserve								
	24-Mar-2009 J.M. Scriber								
Papilio canadensis	VERMONT Bennington Co.					3			MSU
Canadian Tiger Swallowtail	Sandgate Road								
	17-Jun-2009 H. Romack								
Papilio canadensis	MICHIGAN Cheboygan Co.						3		MSU
Canadian Tiger Swallowtail	Mullet Lake								
	1-Jun-2007 J.M. Scriber								

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Matthew Aardema

Date 3-Aug-2010

Voucher
No. 2010-06

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

3 August 2010
Curator Date

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