ORIGINAL ARTICLE

The role of latitudinal, genetic and temperature variation in the induction of diapause of *Papilio glaucus* (Lepidoptera: Papilionidae)

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Abstract A key adaptation in insects for dealing with variable environmental conditions is the ability to diapause. The tiger swallowtail butterflies, *Papilio glaucus* and *P. canadensis* are ideal species to explore the genetic causes and population genetic consequences of diapause because divergence in this trait is believed to be a salient factor in maintaining a hybrid zone between these species. Yet little is known about the factors that influence diapause induction in this system. Here we explored how spatial (latitudinal), environmental (temperature) and genetic (hybridization) factors affect diapause induction in this system. Specifically, a series of growth chamber experiments using wild caught individuals from across the eastern United States were performed to: (1) evaluate how critical photoperiod varies with latitude, (2) isolate the stage in which induction occurs, (3) test whether changes in temperature affected rates of diapause induction, and (4) explore how the incidence of diapause is affected in hybrid offspring. We find that induction occurs in the larval stage, is not sensitive to a relatively broad range of temperatures, appears to have a complex genetic basis (i.e., is not simply a dominant trait following a Mendelian inheritance pattern) and that the critical photoperiod increases by 0.4 h with each increasing degree in latitude. This work deepens our understanding of how spatial, environmental and genetic variation influences a key seasonal adaptation (diapause induction) in a well-developed ecological model system and will make possible future studies that explore how climatic variation affects the population dynamics and genetics of this system.

Key words adaptation; critical photoperiod; development; diapauses; facultative; hybridization

Introduction

Adaptations to seasonal variation have allowed insects to inhabit a diversity of environments and synchronize their phenology with resource availability. Key among

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such traits is diapause—the hormonally mediated slowing of metabolic and often physical activity, usually accompanied by reduced morphogenesis and increased resistance to environmental conditions (e.g., changes in food availability, extreme temperatures, or drought) (Tauber & Tauber, 1981). For facultative diapausing insects, the "choice" to diapause or direct develop is induced by environmental cues (Lees, 1956; Tauber *et al.*, 1986; Danks, 1987) such as photoperiod (i.e., changes in day length). In contrast, obligate diapausing insects enter diapause

regardless of environmental cues. This "choice" can substantially impact fitness and has numerous ecological and evolutionary consequences. For example, co-adapted traits such as morphology, host usage and immunological responses may also be affected (Nylin, 2013). Thus, an understanding of how environmental and genetic factors influence diapause induction is critical to predicting species- and population-level responses to environmental change.

The North American hybrid zone between 2 parapatric butterfly species, the eastern tiger swallowtail, *Papilio glaucus*, and the Canadian tiger swallowtail, *P. canadensis*, is an ideal system to explore how geographic variation in climate and diapause can interact to influence the ecology and evolution of insects. These species are estimated to have diverged ∼0.6 million years ago (Putnam *et al.*, 2007; Kunte *et al.*, 2011; Cong *et al.*, 2015). While it is unclear what initially drove divergence between these species, climate appears to be a prominent factor in maintaining the location of the hybrid zone (Scriber *et al.*, 2003; Scriber, 2011) where the northern range boundary of *P. glaucus* and southern range boundary of *P. canadensis* overlap from Minnesota to New England in the United States (Rothschild & Jordan, 1903; Scriber, 1990). This species boundary (and hybrid zone) coincides with changes in the length of the growing season and a switch from facultative diapause in *P. glaucus* to obligate diapause in *P. canadensis*.

The different diapause strategies (obligate vs. facultative) appear to be differentially selected for across the hybrid zone, acting as a strong isolating barrier between the 2 species (Scriber *et al.*, 2014). In butterflies, the sex chromosomes are Z & W and females are the heterogametic sex (i.e., female are ZW and males ZZ). *P. canadensis* has a Z-linked factor that results in photoperiodically insensitive "obligate diapause" (Rockey *et al.*, 1987a). This factor is probably recessive (Hagen & Scriber, 1989). In contrast, *P. glaucus*, is capable of multiple generations throughout its range as it is a "facultative" diapauser. The nearly complete lack of introgression in mtDNA and Z-linked genetic markers associated with diapause across the hybrid zone provides some evidence that selection on diapause is restricting gene flow between these species (Hagen & Scriber, 1989). Yet, despite the important role diapause plays in the population dynamics and genetics of this system, surprisingly little is known about the factors that induce diapause in these species.

In order to take advantage of this ecological model system to explore how seasonal adaptations such as diapause interact with climatic variation to influence the population dynamics and genetics of these species, it is necessary to have a deeper understanding of how environmen-

tal and genetic factors affect the induction of diapause in these butterflies. While facultative diapause in *P. glaucus* appears predominantly induced by changes in photoperiod (Hagen & Lederhouse, 1985; Rockey *et al.*, 1987b), temperature may also alter this response, as has been observed with many other Lepidopteran species (e.g., Way & Hopkins, 1950; Gomi, 1997; Xu *et al.*, 2014). Similarly, critical photoperiod has been shown to vary by latitude (Danilevskiĭ, 1965; Lankinen & Lumme, 1984) and altitude (Bradshaw, 1976) in many species of diapausing insects. In *P. glaucus*, how critical photoperiod varies across its range is not known. The stage in which diapause is induced is also unclear for *P. glaucus*, yet knowledge of which stage is sensitive to environmental cues will be necessary to model its development (Stoeckli *et al.*, 2012). Similarly, deeper insights into how genetic variation (e.g., hybridization) influences diapause induction will be useful for elucidating the genetic architecture of this trait and the effects of introgression on the population dynamics and genetics of this tiger swallowtail hybrid zone.

Here we set out to test 4 hypotheses related to how environmental and genetic variation influence the induction of diapause in these ecological model organisms. Specifically, we tested the hypotheses that (1) critical photoperiod will increase with increasing latitude in *P. glaucus*, (2) the sensitivity to changes in photoperiod (diapause induction) will occur in the larval stage of *P. glaucus*, (3) warmer temperatures during the larval stage will significantly reduce the incidence of *P. glaucus* diapause, and (4) hybrid offspring from ♀ *P. canadensis* × ♂ *P. glaucus* crosses will exhibit an incidence of diapause similar to that of the father (*P. glaucus*).

Materials and methods

General rearing practices for all experiments

We collected female butterflies in the field from populations described in each experimental section below. These females were assumed to have mated in the wild unless specified otherwise. We allowed females to oviposit in the lab inside clear plastic or wire-mesh arenas placed next to a light source and with host plant leaves inside. On a daily basis, we fed the females with a 20%–25% honey water solution and collected all eggs. Larvae (initially in groups of 5 neonates per dish) were reared starting from the second instar individually in 15 × 2.5 cm (diameter × height) Petri dishes with host plant leaves from the field. For populations collected in Florida, larvae were reared to pupation on sweet bay (*Magnolia virginiana* L. of the Magnoliaceae), while all other larvae in each

experiment were reared on black cherry (*Prunus serotina* Ehrh. of the Rosaceae). Upon pupation, we placed the pupae inside a screen cylinder, which was kept inside its specific chamber treatment until adult emergence.

Latitudinal variation in critical photoperiod

We collected adult *P. glaucus* females in Highlands and Levy counties of Florida (27–29°N latitude; 22 families), Adams, Gallia, and Lawrence counties of Ohio (38–39°N latitude; 19 families) and St. Joseph and Clinton counties of Michigan (42–43°N latitude; 16 families) between 1993 and 1995. Eggs were placed into an environmental control growth chamber with a long-day photoperiod (18 : 6 h light : dark at constant 22 or 25 °C) and hatching larvae were then kept at a constant 25 °C for the remainder of the experiment. We randomly allocated the larvae from each family to 1 of 9 photoperiod treatments (12 : 12, 13 : 11, 13.5 : 10.5, 14 : 10, 14.5 : 9.5, 15 : 9, 15.5 : 8.5, and 16 : 8 h; light : dark cycle). Due to previous direct development of some Florida pupae under 14 : 10 (light : dark cycle), "short" days, Florida rearing experiments were also done under several additional shorter photoperiods (8 : 16, 9 : 15, 10 : 14, 11 : 13, and 12 : 12 h; light : dark cycle). The average development from pupae to eclosion is generally less than 3 weeks at 25 °C (Scriber *et al.*, 2002; Scriber *et al.*, 2008). Therefore, after 6 weeks we assumed live pupae were in diapause and discarded dead pupae (those that were stiff and/or hallow). The average sample size for each latitude × photophase combination was ∼13 individuals (*n* = 345), generated from 22, 19, and 16 families from Florida, Ohio, and Michigan respectively (Table 1).

To determine the critical photoperiod for each latitudinal region, we performed logistic regressions for each sampling location (∼28, ∼38.5, and ∼42.5°N). Regressions were fit using the glm function from the stats package (v3.0.2) in the R environment (v3.1.2), using a quasi-binomial distribution and the logit link function. Critical photoperiod was estimated using the coefficients from each logistic regression to determine the photophase at which 50% of individuals were estimated to have diapaused.

Stage of diapause induction

Prior to this study, the stage of diapause induction—the developmental stage where an individual becomes sensitive to environmental cues that trigger diapause—was not known. To determine the stage of diapause induction, we placed eggs from wild caught *P. glaucus* females (Jackson Co. IN, USA; 39°N latitude) during spring 2014, into 1

Table 1 Sample sizes for different latitudinal regions under a range of photophase treatments.

Latitude	Photophase	*n*
28	8	10
28	9	10
28	10	9
28	11	7
28	12	13
28	13	13
28	13.5	4
28	14	12
28	14.5	9
28	15	5
28	15.5	7
28	16	–
38.5	8	–
38.5	9	–
38.5	10	–
38.5	11	–
38.5	12	26
38.5	13	3
38.5	13.5	17
38.5	14	19
38.5	14.5	17
38.5	15	21
38.5	15.5	23
38.5	16	13
42.5	8	–
42.5	9	–
42.5	10	–
42.5	11	–
42.5	12	19
42.5	13	9
42.5	13.5	7
42.5	14	8
42.5	14.5	8
42.5	15	21
42.5	15.5	10

of 9 treatments that differed in light : dark cycle and were kept at constant 30 °C. Individuals were moved from long-day (16 : 8 h light : dark cycle) to short-day (12 : 12 h light : dark cycle) conditions after 0 (date of hatch; always short day), 5, 10, 15, 20, and 25 d of larval development and when they reached the "wandering" (or prepupal stage) and pupal stage. To estimate the background rate of diapause under long-day conditions (including false positives due to mortality), a group of individuals were kept in long-day (16 : 8 h light : dark cycle) conditions throughout the entire experiment. We then compared the proportion

of individuals that direct developed versus diapaused to determine the stage of development at which diapause was initiated (i.e., the stage where diapause incidence fell below 50%).

Temperature and diapause induction

Temperature has been shown to alter incidence of diapause in many insect orders, including lepidoptera (Hodek & Hodková, 1988; Bale & Hayward, 2010; Saunders, 2014). Here, we tested the hypothesis that warmer temperatures during rearing (larval stage) would significantly reduce the incidence of diapause. Eggs from wild caught *P. glaucus* females from Adams Co., OH (38.5 °N lattide; 4 families; collected July 7–9, 1985) were reared under a range of photoperiods that span the critical photoperiod (3 photoperiods of 16 : 8, 15 : 9, and 14 : 10 h light : dark cycle) for induction and range of temperatures (4 temperatures of 20, 22.5, 25, and 27.5 °C) that larvae would likely experience in the field, for a total of 12 different growth chamber treatments. Fisher's Exact tests were performed using the package RVAideMemoire (v0.9.50) from the R environment (v3.1.2) to determine whether the incidence of diapausing individuals significantly differed between temperature treatments within a photoperiod treatment.

Hybridization and diapause induction

To determine whether diapause induction is altered in hybrids, we made F1 hybrid families from *P. canadensis* × *P. glaucus* (mother × father) pairings and randomly split the resulting offspring between 2 treatments that differed only in photoperiod—a "short-day" (12 : 12 h light : dark cycle) and "long-day" (18 : 6 h light : dark cycle) treatment. The temperature of both treatments was the same at a constant 22 °C. The reciprocal cross—F1 hybrids from *P. glaucus* × *P. canadensis*—was not used because this cross produces no facultative diapausing female offspring and only half of the male offspring are facultative diapausers as diapause induction is Z-linked (females: ZW, males: ZZ).

Wild captured *P. glaucus* males from Lawrence Co. Ohio (collected in 2008) were hand-paired to virgin (lab reared) *P. canadensis* females to form hybrid pairings. The mothers of these virgin females were collected in 2007 from Bennington Co. Vermont. We used wild caught males as they have higher fertility than lab-reared males (Lederhouse *et al.*, 1990). Our hybrid pairings resulted in 8 families of F1 hybrids. Similar procedures for oviposition, larval rearing, and pupal storage were used as the parental populations described above, with the exception that these hybrid larvae were initially reared in small groups of 2–3

individuals. A Goodness of Fit test of independence was performed to determine whether the sex ratio of direct developing individuals was significantly different between photophase treatments using the package RVAideMemoire (v0.9.50) from the R environment (v3.1.2).

Results

Latitudinal variation in critical photoperiod

As expected, critical day length (CDL) (estimated as the day length at which incidence of diapause is 50%) increased with increasing latitude for split broods of *P. glaucus* (Fig. 1; Table 2). CDL was estimated to be ∼10, ∼14.6, and ∼15.9 h for the Florida (28°N latitude), Ohio (38.5°N latitude), and Michigan (42.5°N latitude) populations, respectively.
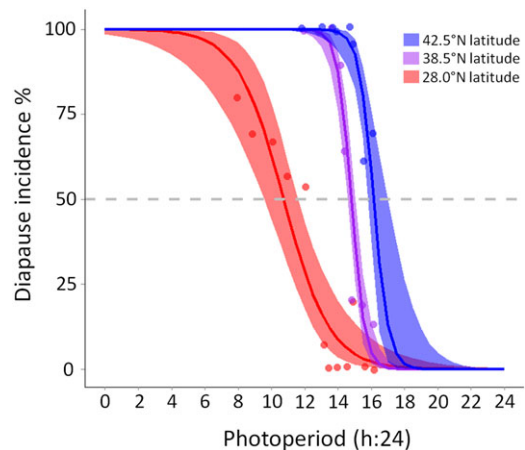

Fig. 1 Photoperiod response curves for the production of diapausing *P. glaucus* pupae at 25 °C from different latitudes across the species' range. The critical day length is the photoperiod at which 50% (dashed gray line) of individuals underwent diapause. The different colors represent the different populations (latitudes) sampled.

Table 2 Regression coefficients and significance levels of incidence of diapause by latitude.

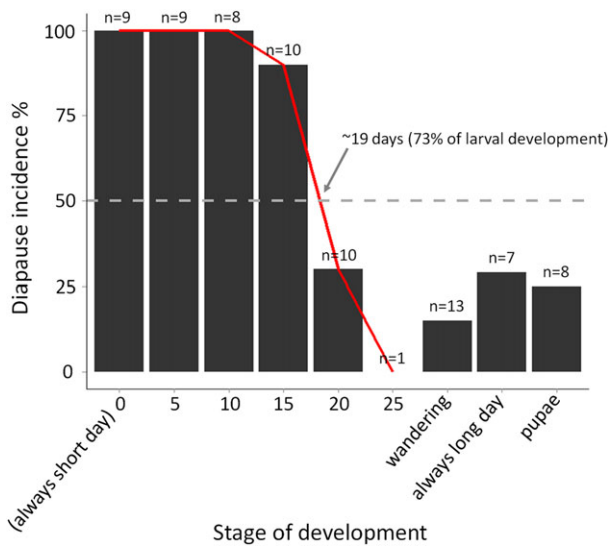| Latitude	Parameter	Estimate	Std. error	*t* value	Pr(>|*t*|)
28	Intercept	7.51	1.83	4.12	0.00
	Photophase	−0.70	0.16	−4.46	0.00
38.5	Intercept	29.04	5.34	5.43	0.00
	Photophase	−1.96	0.36	−5.41	0.00
42.5	Intercept	36.19	9.90	3.66	0.01
	Photophase	−2.25	0.64	−3.54	0.01

Fig. 2 Proportion of *P. glaucus* individuals entering diapause by photoperiod treatment. For treatments 0, 5, 10, 15, 20, and 25 larvae were moved from long day (16 : 8 h light : dark cycle) to short day (12 : 12 h light : dark cycle) after 0 (date of hatch; always short day), 5, 10, 15, 20, and 25 d, respectively. For the "wandering," "pupae" and "always long day" individuals were moved during the wandering or pupal stage, or kept in long day the entire treatment, respectively. All treatments were kept at constant 30 °C. Sample sizes are given above each treatment.

Stage of diapause induction

In line with our predictions, diapause induction was found to occur during the larval stage of development. Larvae moved from short to long-day conditions between 15–20 d showed the greatest change in the incidence of diapause, and this was the developmental period at which the incidence of diapause crossed the 50% threshold (Fig. 2). Specifically, induction was estimated to occur in the later stages of larval development—at ~73% of the way through larval development (~4th and 5th instar; ~19 d through the average 26 d of development).

Temperature and diapause induction

Contrary to our prediction, temperature was not found to significantly affect the incidence of diapause in *P. glaucus* individuals (Fig. 3). Within each photoperiod treatment, there was no significant difference in the proportion of diapausing *P. glaucus* individuals between any of the 4 (20, 22.5, 25, and 27.5 °C) temperature treatments (Fisher's Exact Tests; $P > 0.05$). Similar to the results from the *Latitudinal variation in critical photoperiod* experiment, the Ohio populations (38.5°N) used in this ex-

periment were also found to have a CDL falling between 14 and 15 h.

Hybridization and diapause induction

The prediction that short-day conditions would increase the incidence in diapause (decrease proportion of individuals direct developing) in *P. canadensis* × *P. glaucus* hybrids was only partially supported. While short-day conditions did increase the incidence of diapause of offspring overall, the effect were dependent on the sex of the offspring (Fig. 4). Under long-day conditions (18 : 6 h light : dark cycle) nearly all pupae directly developed, with a roughly equal proportion of females (54%; $n = 104$) and males (46%; $n = 88$) (Fig. 4); only a single pupae diapaused (0.05% of 193 pupae). In contrast, under short-day conditions (12 : 12 h light : dark cycle), the proportion of direct developing pupae dropped to 37% (213/572). Of the pupae that direct developed, the proportion of those that were females was significantly greater ($G = 45.4$; $P < 0.0001$), with 31% (180/213) females still directly developing while only 6% (33/213) of males direct developed. For comparison, the 37% of *P. canadensis* × *P. glaucus* pupae (31% being female) that direct developed under short-day conditions contrasts with the 0% of *P. glaucus* (parental species that exhibits facultative diapause) pupae that direct developed under the same photoperiod (12 : 12 h light : dark cycle) and temperature (25 °C) conditions (Fig. 2).

Discussion

Diapause is a pivotal factor for latitudinal and seasonal adaptation in many insects. Here we provide an evaluation of how spatial (latitudinal), environmental (temperature), and genetic (hybridization) variation affect diapause induction in the facultative diapausing species *P. glaucus*. As predicted, the critical photoperiod to induce diapause increased with increasing latitude for populations spanning the range of *P. glaucus*. This result is in line with many other studies of facultative diapausing temperate insect species where critical photoperiod has been found to increase with distance from the equator (Danilevskiĭ, 1965; Lankinen & Lumme, 1984), including the spicebush swallowtail butterfly, *Papilio troilus* (a closely related species in a sister group to *P. glaucus* and *P. canadensis*) (Valella & Scriber, 2002). Changes in critical photoperiod has been argued to be a common evolutionary response to climate change (Bradshaw & Holzapfel, 2007) among insects and has been shown to evolve rapidly in response to changes in climate (Bradshaw & Holzapfel, 2001). Given
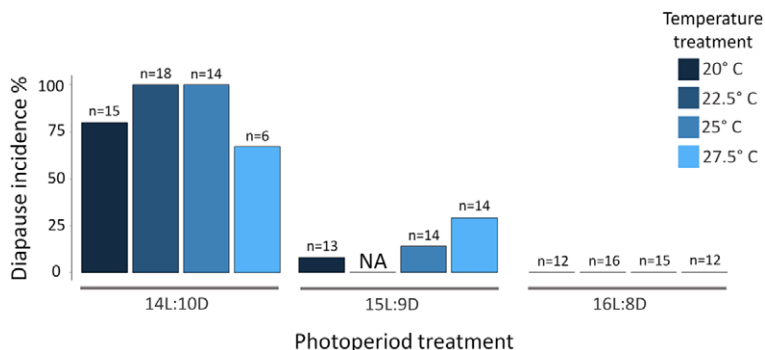
Fig. 3 Incidence of diapause for *Papilio glaucus* reared from neonate larvae to pupation under three different photoperiods and four constant temperatures. No significant differences were found between temperature treatments within each photoperiod treatment (Fishers exact tests; $P > 0.05$). NA: the growth chamber was not functioning properly for the first 5 d of neonate larval growth (total darkness) so were excluded from analyses. Sample sizes are given above each treatment.
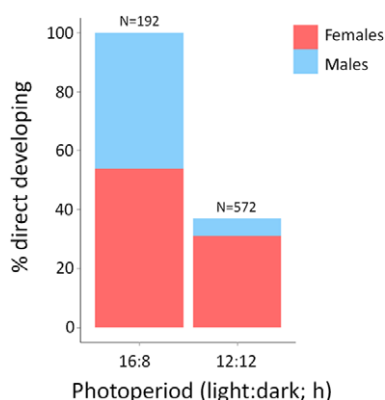

Fig. 4 Proportion of direct developing hybrid ($♀$ *P. canadensis* × $♂$ *P. glaucus*) pupae reared under different photophase conditions. There was a significant difference in the proportion of males and females direct developing between photophase treatments ($G = 160$; $P < 0.0001$). Sample sizes are given above each treatment.

that our estimates of critical photoperiod were measured in 1993–1995 and there has been documented warming in Wisconsin, it will be important and interesting to repeat this study to determine whether critical photoperiod is changing as a response to recent climatic warming in the eastern United States (Kucharik *et al.*, 2010; IPCC, 2014).

Similarly, critical photoperiod often increases with increasing altitude (Bradshaw, 1976). Future studies exploring how critical photoperiod varies by altitude in *P. glaucus,* may help determine what role, if any, variation in critical photoperiod may be playing in the formation of the purported hybrid species—*P. appalachiensis*—that only occurs in the higher altitudes of the Appalachian Mountains (Pavulaan & Wright, 2002; Scriber *et al.*, 2008; Ording *et al.*, 2010; Kunte *et al.*, 2011). It is believed that the intermediate eclosion phenology of this putative hy-

brid species is producing allochronic isolation between hybrids and parental species (Kunte *et al.*, 2011). However, it is possible that diapause induction of *P. glaucus* populations at these higher latitudes may also be contributing to temporal isolation, as it would result in fewer individuals having a second generation and in turn reduce the likelihood that *P. glaucus* individuals backcross with hybrids that exhibit later seasonal phenology. Studies evaluating critical photoperiod in these higher altitude populations across the Appalachian Mountains could help to test this hypothesis.

For many insects, the stage in which diapause is induced (i.e., developmental stage sensitive to changes in photoperiod) usually precedes the stage in which diapause occurs (Denlinger, 2002). We found this to be the case with *P. glaucus* as well. Evidence from our study suggests that diapause is induced during the larval stage prior to pupation (where diapause occurs), likely near the end of larval development (4th or 5th instar). A sensitive larval stage for diapause is also found in other *Papilio* species—*Papilio troilus* (Deering *et al.*, 2003), *Papilio polyxenes* (Oliver, 1969), and *Papilio machaon* (Shimada, 1983) along with other Lepidopteran species such as *Helicoverpa armigera* (Kurban *et al.*, 2007), *Hyphantria cunea* (Masaki *et al.*, 1968), and *Pieris rapae* (Barker *et al.*, 1963).

Temperature has been shown to influence diapause in many insect species (e.g., Menaker & Gross, 1965; Kobayashi & Numata, 1995; Wang *et al.*, 2014). However, in contrast to our prediction, temperature does not appear to affect the incidence of diapause in *P. glaucus*. It is possible that the range of temperature variation used in our study was too moderate and that more extreme temperatures (i.e., <20 °C and >30 °C) would result in more measurable changes in the incidence of diapause (Wang *et al.*, 2009). In our experiment to determine the stage

of diapause induction we used 30 °C. While this experiment was not designed to test for the effect of temperature specifically, our results suggest that even at 30 °C there is no effect of temperature on diapause induction—that is, 100% of individuals still diapaused when kept in constant short-day conditions. Conversely, ~25% of individuals kept in long-day conditions throughout development were phenotyped as having entered diapause. While this result might suggest that higher temperatures can lead to an increased incidence of diapause, the more likely explanation is that this reflects small sample sizes (with $n = 10$, one individual can lead to a 10% change) and bet-hedging—a proportion of facultative diapausing insects often enter diapause even under strongly nondiapause promoting conditions (Hopper, 1999). This observation could also reflect background mortality (individuals falsely scored as diapaused), but given that we monitored pupae for 6 weeks after pupation and dead pupae are easy to identify (turn black or the abdomen becomes rigid) this possibility is less likely. It is also possible that the populations used in our experiment were less sensitive to temperature and that sensitivity to temperature during diapause induction varies across the species range. For example, diapause induction was more sensitive to temperature in trivoltine than bivoltine populations of the fall webworm, *Hyphantria cunea* (Gomi, 1997). Studies looking at clinal patterns of sensitivity to both critical photoperiod and temperature are rare, but are important for understanding how insects respond to climatic changes across their range.

Diapause induction is known to be Z-linked in *P. glaucus* and appears to be controlled by a few genes (Hagen & Scriber, 1989). A close relative, the pale tiger swallowtail butterfly, *P. eurymedon*, from the western United States also has obligate diapause, and based on hybrids of these females with *P. glaucus*, facultative diapause (od–) appears to be recessive (West, 1995). Thus, our finding that many *P. canadensis* × *P. glaucus* hybrid adults emerged under the short-day photoperiod conditions was a surprise, as we expected the majority of these individuals to have diapaused. This was especially unexpected in Pc × Pg hybrid daughters, which are all presumably hemizygous with a *P. glaucus* Z-chromosome. We had expected to see all hemizygous (for a Z-linked od+ allele) Pc × Pg hybrid daughters and at least half of the heterozygous sons enter pupal diapause at the 12 : 12 h light : dark cycle, especially since all *P. glaucus* fathers were from the same Ohio population where all pupae (from 19 different families) diapaused at every photoperiod less than 14 h. Perhaps the gene on the Z-chromosome from the facultatively diapausing Ohio *P. glaucus* (od–) is not as dominant as the obligate diapause gene (od+) from *P. canadensis* fathers. Although the expression of the Z-linked *od–* allele (if

recessive) would be stronger in hemizygous (ZW) daughters, the reason why so many of these hybrid females directly developed under short-day conditions remains unclear.

Among potential alternative explanations, maternal effects could be playing a role (Mousseau, 1991) or the observed, unexpected diapause incidence could be due to the presence of autosomal, mitochondrial or other sex-linked modifiers of the Z-linked facultative diapause response gene (od–). Genetic control of diapause in insects often involves both sex-linked and autosomal loci (Tauber & Tauber, 1981), and potential epistatic interactions between *P. glaucus* Z-chromosome and *P. canadensis* autosomes may prevent larvae/pupae from successfully entering diapause, as was described for 2 *Colias* species and their hybrids (Karowe, 1994). Another possible explanation is that seasonal photoperiodism is under relaxed selection given that obligate diapausers are no longer using photoperiodic cues to enter diapause (diapause occurs regardless of day length). Thus genes involved in photoperiodism could drift in either direction in *P. canadensis* resulting in an increase or decrease in critical photoperiod. The consequences of this neutral evolution would not manifest phenotypically in *P. canadensis*, but could result in aberrant photoperiodism thresholds in hybrids of this species and *P. glaucus*. In any case, these and other possibilities need to be investigated in the future to resolve the mechanism driving this unexpected finding.

In conclusion, our study provides greater insight as to the genetic, developmental and environmental factors related to diapause induction in what is an excellent model system for exploring the population genetic consequences of diapause induction. Specifically, we found that induction occurs in the larval stage, does not appear to be sensitive to temperature and appears to have a complex genetic basis (i.e., is not simply a dominant trait following a Mendelian inheritance pattern). This work is useful for understanding how diapause drives divergence in this system and provides key life history data that when combined with previous studies on development can be used to model how climatic variation affects the population dynamics and genetics of these hybridizing species.

Acknowledgments

Disclosure

The authors declare that there is no conflict of interest.

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