

Hot summer temperatures may stop movement of *Papilio canadensis* butterflies and genetic introgression south of the hybrid zone in the North American Great Lakes Region

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The reasons that the northern tiger swallowtail butterfly *Papilio canadensis* does not move south of the Great Lakes hybrid zone, where it meets *P. glaucus*, may be largely due to natural temperature-induced stress on diapausing pupae. Temperatures of 36°C for only four days killed all *P. canadensis* (from northern Michigan) and most of the *Papilio troilus*, a species that lives south of the hybrid zone (sympatrically with *P. glaucus*). In contrast, interspecific hybrids (*P. glaucus* mother \times *P. canadensis* father) had significant adult eclosion or pupal survival at both 30° and 36°C. All surviving hybrid pupae were only females (the heterogametic sex, which are known to express the prolonged diapause due to Haldane effects).

The southern species, *P. troilus*, had almost all (81%) normal (non-deformed) adults eclose at 30°C, whereas only 20% of the *P. canadensis* females and ca 30% of the *P. canadensis* males eclosed to produce non-deformed adults. Unlike the case with *P. troilus* and *P. canadensis*, no hybrid females eclosed at 30°C (only males did). Unlike *P. troilus* and *P. canadensis* pupae, hybrid (female) pupae remain viable, some of which have already successfully emerged after the chamber experiments.

A follow-up study using *P. glaucus*, *P. canadensis* (from Vermont), and their hybrids with more normal lower thermal regimes included (27°, 30°, 33°, and 36°C) again showed higher hybrid survival as unclosed (living) pupae at 36°C. In addition, *P. glaucus* and *P. canadensis* showed high mortality and wing deformity of eclosing adults at 36°C, suggesting that geographic source of the *P. canadensis* may reflect differential tolerances of the extreme 36°C temperature.

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The Great Lakes of North America hybrid zone/ecotone

A butterfly hybrid zone between the Canadian *Papilio canadensis* R & J and the eastern *P. glaucus* L. tiger swallowtails has remained relatively constant in its geographic location (41°–44°N latitude) across the Great Lakes region for more than a century (Merriam 1894, Scriber and Lederhouse 1992, Scriber 1996a, Scriber et al. 1996). Periods of regional warming, reflected during the summer growing season by increased seasonal thermal unit (degree day) accumulations have permitted a

second generation to be successful, allowing periodic northward movement of *P. glaucus* individuals and genetic introgression north of this hybrid zone (Scriber and Gage 1995). This trend has been noted elsewhere as well (Parmesan et al. 1999, Hughes 2000).

A single very cold year, a late spring freeze, or an early autumn freeze (such as 1992) can have a severe impact on *Papilio* survival and geographic range limits even if it is only once in 10 yr (Ayres and Scriber 1994, Scriber and Gage 1995, Scriber 1996b, Tesar and Scriber 2001). In fact, the lack of cryoprotectants and increased pupal mortality of *P. glaucus* relative to *P.*

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canadensis at extremely cold temperatures may partially explain why *P. glaucus* does not appear further north, across Canada and into Alaska (Kukal et al. 1991).

The factors that limit gene flow southward from the Canadian tiger swallowtail species (*P. canadensis*) basically remain unidentified (Scriber 1996a). As a polyphagous species, able to eat dozens of tree species from the favored plant families (Salicaceae, Betulaceae, Rosaceae, Oleaceae; Scriber 1984), *P. canadensis* is not confined to boreal habitats of Canada and Alaska by suitable host plant distribution alone (Scriber 1988). Ash (*Fraxinus* spp.) and cherry (*Prunus* spp.) tree hosts exist throughout the eastern half of the USA southward into central Florida (Scriber 1986a, Scriber and Lederhouse 1992). However, there is significantly limited introgression of *P. canadensis* traits south of the Great Lakes hybrid zone including: morphological (adult wing patterns and larval traits; Luebke et al. 1988, Scriber 1990); biochemical (detoxification abilities and diagnostic alleles; Scriber 1986b, Hagen et al. 1991), physiological (facultative and obligate diapause; Rockey et al. 1987a, b) and ecological factors (oviposition preferences and mimicry polymorphisms; Scriber 1988, Bossart and Scriber 1995, Scriber et al. 1996).

What stops individual movement and gene flow of *P. canadensis* south of the hybrid zone?

We hypothesized that a primary reason constraining introgression of *canadensis* genes southward may be poorer survival of obligately diapausing *P. canadensis* pupae at higher temperatures during the period of summer which occurs with increasing lengths progressively south of the hybrid zone in the eastern USA. Such hypothesized intolerance of higher temperatures for extended periods derives from the presumed origin of the species in Beringian refuges of North America (corresponding to Alaska during the Pleistocene glaciation) (Scriber 1988, Scriber et al. 1991). Even in a normal year, July and August temperatures south of the Great Lakes area could exert heat stress for such a boreal butterfly species as *P. canadensis*. For example, the heat wave mid-July 1999 produced 36°C temperature highs most of the period from mid-July to mid-August across the corn belt from the Dakotas to Georgia (Anon. 1999). During the summers of 1998 and 1999, three to five weeks of consecutive > 37°C daily temperature highs were observed across Texas and many areas of the southeastern USA. At no point did the areas north of the hybrid zone in Wisconsin, Michigan, New York and Ontario, Canada experience temperatures exceeding 35°C (Fig. 1), and rarely did they exceed 30°C (Anon. 1999).

As *P. glaucus* pupae are less well adapted to extreme cold encountered by *P. canadensis* in the north (Kukal et al. 1991), so too we wondered if *P. canadensis* pupae

were correspondingly poorer at tolerating high summer temperatures usually encountered by *P. glaucus* south of the Great Lakes hybrid zone. Our preliminary studies of diapause termination at 14°, 20° and 26°C showed progressively greater pupal survival and faster pupal diapause termination at higher temperatures for *P. canadensis*, *P. glaucus*, and their hybrids (Scriber et al. unpubl.; see also Table 5). However, *P. glaucus* and hybrid females failed to emerge or died at 14°C. A similar study of diapause termination in *Papilio troilus* (Deering et al. 2001), showed very similar results; with no adult emergence at 14°C regardless of length of the photoperiod (18:6 or 12:12 h).

We predicted that at the other end of the temperature range we might see poorer survival of the northern boreal-adapted *P. canadensis* pupae. Since Lepidoptera sometimes respond to rainfall as cues for diapause termination, we conducted this diapause termination study with and without direct daily watering to simulate rain. We used diapausing pupae of *P. canadensis* and diapausing hybrids of *P. glaucus* and *P. canadensis* at 30° and 36°C to test this thermal stress hypothesis. Although we had no southern *P. glaucus* pupae in diapause available for our study, we did have diapausing spicebush swallowtail *P. troilus* pupae from the same southern (heat-adapted) geographic sources as *P. glaucus* inhabit.

Methods

Sources of diapausing pupae

Diapausing pupae of *P. troilus* (n = 79 from three families) were reared in 1998 from mothers collected in St. Charles Co., Missouri. *Papilio canadensis* pupae (n = 292 from 47 families) were reared in 1998 from mothers collected in northern Michigan (Cheboygan, Dickinson, Otsego, and Leelanau Co.) and Wisconsin (Florence Co.). Hybrid pupae (n = 72 from 12 families of *P. glaucus* females mated to *P. canadensis* males) were similarly reared in 1998, stored as diapausing pupae in total darkness at 3–5°C until we brought them out and divided them into the four treatments of this study. We had not saved hybrid pupae of the reciprocal pairing for this study. We allowed two days at room temperatures (23°–25°C) after removal from the cooler, before we set them up in the replicated 30° and 36°C chambers.

The pupae were individually placed in Petri dishes (150 mm diam.) with wire screening (to allow room for the butterfly to expand its wings at emergence) and then randomly distributed (noon of 8 July) among two temperatures, 30° and 36°C in two replicate chambers. The photoperiod was maintained constant at 18:6 (light:dark). The temperatures of the chambers were monitored and recorded twice a day to maintain con-

stancy. Half of the total number of each species were sprayed with room temperature tap water twice a day at ca 9:00 AM and 4:15 PM. The water sprays continued until 25 July (total of 18 d) by which time most eclosions had occurred.

Since we were not sure how lethal these two temperatures (30° and 36°C) would be for pupae, we removed a subset of *P. canadensis* pupae from both the watered and non-watered treatments from each of the four chambers after only four days. This was also done for

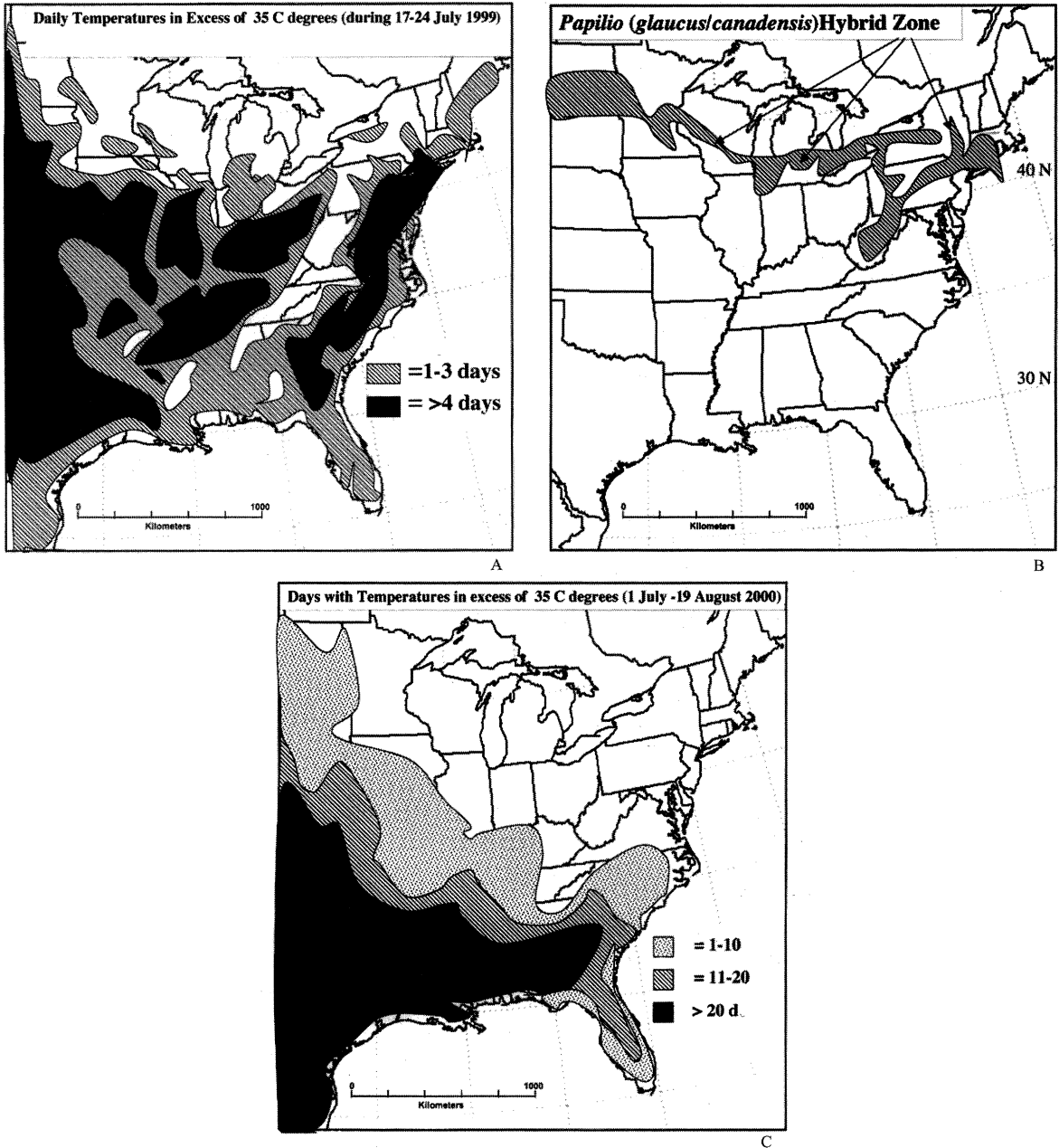


Fig. 1. A) During the eight days between 17 and 24 July 1999, the temperature exceeded 35°C (95°F) across most of the eastern USA south of the Great Lakes region of North America. The daily high was > 35°C for 4–8 d (darkest shade), 2–4 d (lightly shaded) and 0–1 d (across the Great Lakes region, New England and Appalachian Mountains). The following week of 1999 was even hotter (> 39°C in much of the same shaded areas). B) The Great Lakes hybrid zone between *Papilio canadensis* and *P. glaucus* is delineated by the dark band corresponding to seasonal mean degree day accumulations of 1400–1500°C (base 10°C). C) Number of days 1 July through 19 August 2000 with daily maximum temperature 95°F/35°C or greater (modified from Anon. 2000 USDA/NOAA Agricultural Statistics). This was a cooler summer.

Table 1. Post-diapause development and adult eclosion (in 1999) of *P. canadensis*¹, hybrid (*P.g.* × *P.c.*) and *P. troilus* under two temperatures (30° and 36°C; 16:8 photoperiod).

		Eclosed adults		Unclosed pupae ²		
	Total (n)	Normal	Deformed/ partial	Dead (with imago developed)	Dead (no imago)	Alive
<i>P. canadensis</i>						
(males 30°C)	(46)	(13) 28.3%	(21) 45.7%	(8) 17.4%	(1) 2.2%	(0) 0.0%
(females 30°C)	(49)	(10) 20.4%	(22) 44.9%	(10) 20.4%	(7) 14.3%	(0) 0.0%
(males 36°C)	(52)	(0) 0.0%	(1) 1.9%	(41) 80.4%	(9) 17.3%	(1) 1.9%
(females 36°C)	(36)	(0) 0.0%	(1) 2.8%	(28) 77.8%	(5) 13.9%	(2) 5.6%
Hybrid (<i>P.g.</i> × <i>P.c.</i>)						
(30°C)	(36)	(10) 27.8%	(9) 25.0%	(4) 11.1%	(3) 8.3%	(10) 27.8%
(36°C)	(36)	(0) 0.0%	(1) 2.8%	(15) 41.7%	(6) 16.7%	(14) 38.9%
<i>P. troilus</i>						
(30°C)	(43)	(35) 81.4%	(2) 4.7%	(3) 7.0%	(3) 7.0%	(0) 0.0%
(36°C)	(36)	(1) 2.8%	(4) 11.1%	(31) 86.1%	(0) 0.0%	(0) 0.0%

¹ Male *P. canadensis* were also analyzed after only four days and eight days at these elevated temperatures (see Table 2). At 36°C the surviving *P. canadensis* died within 40 d after the chamber experiment.

² Of the unclosed hybrid pupae, there were 8 males and 8 females at 30°C; and 18 males and 17 females at 36°C, which is an equal sex ratio. However, more of the dead (unclosed) hybrid pupae were males and 17 of the 17 eclosing adults were male, leaving 8 live females and 2 live males at 30°C and leaving 12 live females and 1 live male at 36°C to potentially emerge the next year. In fact, four of these hybrid females did emerge 40–80 d after the chamber experiments were terminated (8 August 1999). At 80 d post-experiment the males were all dead and the only survivors were viable hybrid females (7 from 30°C, 6 from 36°C).

another subset at eight days. These water and non-watered treatments were continued in this holding room, which was in constant light and ranged from 22° to 25°C.

We collected emerged (or partially emerged) adults daily and recorded dates and times of eclosion. We also measured forewing lengths for normal (non-deformed) individuals, and verified sex by examining the genitalia. At the end of the study (8 August 1999) the remaining pupae were examined to see if they were alive or dead. We left live pupae in the lighted room at 22°–25°C for five more weeks, during which time only one hybrid female emerged (from 30°C chamber #1). The small number of live pupae were returned to diapause storage (4–5°C total darkness) for other studies.

During the spring of 2000 (after the results of our 30° and 36°C study were complete), we set up a follow-up study with four thermal regimes (27°, 30°, 33°, and 36°C) in order to evaluate more normal thermal regimes and also to include *P. glaucus*. We water misted all pupae in the second study (since no significant differences emerged in water sprayed or unsprayed treatments in our first study). The same growth chambers and procedures were used in this study, however, we used *P. canadensis* from another population (Bennington Co., Vermont) and *P. glaucus* (from Schuylkill Co., Pennsylvania). The same basic stock of diapausing hybrid pupae were used in both studies.

Chamber settings

The chambers were maintained at a 18:6 photo/sco-topphase. Over the four weeks, chamber 1 (set at 30°C) ranged internally from 29.5° to 30.5°C and chamber 3

ranged from 30.5° to 31.5°C. Chambers set at 36°C ranged from 33.5° to 37.0°C (chamber 4) and 35.5° to 36.5°C (chamber 5). Each day, the chamber temperatures were checked and adjusted slightly to desired when the reading was off the desired by one degree or more. The same procedures were used for the eight chambers used in the four temperature follow-up study.

Results

It was very clear (Table 1) that pupae maintained at 36°C temperatures experienced significant (almost total) mortality. At this high temperature, only 13% (n = 36) of the *P. troilus* eclosed. Only 2.8% (n = 36) of the *P. glaucus* × *P. canadensis* hybrids eclosed (with none normal; i.e. all were deformed; Table 1). At this high temperature (36°C), only 2.8% (n = 36; Table 1) of the *P. canadensis* females and 1.9% (n = 52) of the *P. canadensis* males eclosed (all were deformed). Of the unclosed pupae, no *P. troilus* were alive, and for *P. canadensis* only one male and two females were alive. In contrast, 38.9% (n = 14) of the hybrid pupae at 36°C were still alive at the end of the experiment (and after five additional weeks at room temperature). Four of these hybrid females emerged normally at room temperature during the 80 d after the experimental chamber conditions.

The survival was much better for pupae maintained at 30°C. The majority of *Papilio troilus* pupae eclosed (86.1%) and 81.4% were normal (non-deformed) adults (Table 1). Similarly, the majority of *P. canadensis* females (65.3%) and males (79.0%) eclosed. However only 20.4% of the females and 28.3% of the males were successful at producing undeformed adults (Table 1).

Table 2. Eclosion of adults from diapausing pupae at four temperature regimes (all 18:6 photoperiod). These pupae were from Vermont (*P. canadensis*) and Pennsylvania (*P. glaucus*).

		Eclosed adults		Unclosed pupae		Total (n)
		Perfect	Deformed	Dead	Alive	
<i>P. canadensis</i> (Vermont)						
27°C	male	(35) 97.2%	(1) 2.8%	(0) 0.0%	0	36
	female	(31) 93.9%	(0) 0.0%	(2)	0	33
30°C	male	(9) 90.0%	(0) 0.0%	(1) 10.0%	0	10
	female	(7) 77.8%	(1) 11.1%	(1) 11.1%	0	9
33°C	male	(10) 100.0%	(0) 0.0%	(0) 0.0%	0	10
	female	(7) 70.0%	(1) 10.0%	(2) 20.0%	0	10
36°C	male	(0) 0.0%	(6) 66.7%	(3) 33.3%	0	9
	female	(1) 9.1%	(5) 45.5%	(5) 45.5%	0	11
<i>P. glaucus</i> (Pennsylvania)						
27°C	male	(24) 88.9%	(1) 3.7%	(2) 7.4%	(0) 0.0%	27
	female	(23) 85.2%	(2) 7.4%	(2) 7.4%	(0) 0.0%	27
30°	male	(20) 95.2%	(1) 4.8%	(0) 0.0%	(0) 0.0%	21
	female	(12) 92.3%	(1) 7.7%	(0) 0.0%	(0) 0.0%	13
33°	male	(16) 94.1%	(1) 5.9%	(0) 0.0%	(0) 0.0%	17
	female	(13) 76.5%	(3) 17.6%	(1) 5.9%	(0) 0.0%	17
36°	male	(2) 1.8%	(10) 58.8%	(5) 29.4%	(0) 0.0%	17
	female	(0) 0.0%	(6) 35.3%	(10) 58.8%	(0) 0.0%	17
F ₁ hybrids (<i>P.g.</i> × <i>P.c.</i>)						
27°	None used	—	—	—	—	—
30°	males	(5)	(0)	(2)	(10) unknown sex	18 combined sexes
	females	(0)	(0)	(1)		
33°	males	(4)	(0)	(3) unknown sex	(9) unknown sex	17 combined sexes
	female	(1)	(0)			
36°	males	(4)	(0)	(9) unknown sex	(4) unknown sex	18 combined sexes
	females	(0)	(1)			

As with the 36°C treatments, the percent of unclosed pupae that were still alive was very small (0% for *troilus*; 0% for *canadensis* females and 0% for *canadensis* males). In contrast, at 30°C, 27.8% (n = 10) of the unclosed hybrids were still alive, much as observed at the higher (36°C) temperatures.

In all cases, the differences between the growth chamber replications were minimal, and data were pooled for analysis of the durations before eclosion. It is also clear that the mortality was induced before four days in both the 30° and the 36°C chambers.

For the male *P. canadensis* removed from both high temperature chambers to a cooler room, all of the pupae failed to eclose. For the male *canadensis* pupae removed after only four days at 30°, 44.1% eclosed (and 23.5% were normal); for those pupae at 36° removed after four days, only 12.5% eclosed and none were normal adults (Table 3). When compared to continuous rearing of these 30° and 36°C temperatures, it seems that removal after four days did not greatly help survival, suggesting damage was already done.

Table 3. Post-diapause development and adult eclosion of male *Papilio canadensis* under two continuous temperatures (30° and 36°C). Subsets of unclosed pupae were removed from each of four growth chambers at four days.

Male <i>P. canadensis</i>	Total (n)	Eclosed adults		Unclosed pupae		Alive ¹
		Normal	Deformed	Dead (with imago developed)	Dead (no imago)	
[30°C]						
Continuous	(46)	(13) 28.3%	(21) 45.7%	(10) 21.7%	(2) 4.3%	(0) 0.0%
Removed (4 d)	(34)	(8) 23.5%	(7) 20.6%	(15) 44.1%	(2) 5.9%	(2) 5.9%
[36°C]						
Continuous	(52)	(0) 0.0%	(1) 1.9%	(41) 78.8%	(9) 17.3%	(1) 1.9%
Removed (4 d)	(40)	(0) 0.0%	(5) 12.5%	(24) 60.0%	(10) 25.0%	(1) 2.5%

¹ All remaining viable individuals died in the holding room within 40 d of the termination of chamber experiment.

The rain simulation with watering twice daily did not result in earlier eclosion or higher percentage of eclosion or survival (Table 4). The time to adult eclosion from the diapausing pupal state under different temperature regimes is indicated for various *Papilio* taxa (Table 5). It can be generally noted that the emergence is quicker at higher temperatures and that males may be slightly earlier than females (especially in the *P. glaucus* × *P. canadensis* hybrids).

Our follow-up study in 2000 utilized additional temperatures to better assess the "threshold" between 30° and 36°C, and to compare a more normal summer temperature of 27°C. We found very little pupal mortality of *Papilio canadensis*, *P. glaucus*, or hybrids at 27°, 30°, and 33°C temperatures (Table 2). Excellent (normal) adults were observed in 80–100% of the diapausing *P. glaucus* and *P. canadensis* pupae at the three lower thermal regimes (27°, 30°, and 33°C). Mortality increased significantly at 36°C for all three taxa and, furthermore, of those adults that did manage to eclose, most were deformed (Table 2). Once again, about half of the hybrids at 30° and 33°C remained alive but in extended diapause while most non-eclosing pupae at 36°C died.

Discussion

An important thermal selective force is evident in our results showing the inability of 98% of the *P. canadensis* pupae and 97% of the hybrid *glaucus* × *canadensis* pupae, to eclose as functional adults at 36°C. In fact, of those few eclosing, none were normal enough to fly. At 30°C the eclosion to adults was observed for a larger proportion of our experimental subjects (65.3% of female and 79.0% of male *P. canadensis*). However, a high frequency of malformations of the wings were observed in those individuals that did eclose, and many partial eclosions resulted in death of the imago (unsuccessful emergence from the pupal case). Only 20% of the females and 30% of the males that emerged were normal (non-deformed) adults (Tables 1 and 2).

The near-total mortality at 36°C (and very significant mortality at 30°C) for diapausing pupae of *P. canadensis* raises some very important issues relating to southward geographic distribution limits of this univoltine species (Rockey et al. 1987a, Scriber 1988, Scriber and Lederhouse 1992). The long hot summers south of the ecotone delimiting the southern extremes for the boreal forests could be lethal for the *P. canadensis* pupae, even if the larval stages had no trouble completing their

Table 4. Time to adult eclosion (in 1999) at 30°C from diapausing pupae of *P. canadensis*, hybrid (*P.g.* × *P.c.*), and *P. troilus* with and without twice daily water spraying. Data presented as a mean¹ (± SE).

	With water sprays twice daily (n)		Without water sprays (n)	
<i>Papilio canadensis</i>				
Females	(10)	4.6 ± 0.4	(12)	5.5 ± 0.8
Males	(24)	5.0 ± 0.5	(25)	5.2 ± 0.3
Hybrids	(9)	4.7 ± 0.7	(7)	4.9 ± 0.7
<i>P. troilus</i>	(12)	10.2 ± 0.4	(17)	11.0 ± 0.5

¹ No significant differences in days until eclosion between watered and non-watered pupae (t-tests at p = 0.05).

Table 5. Comparison of time to pupal eclosion (mean ± SD) as adults at 30°C (this study) with those at lower temperatures (other studies below). Dash line indicates no study was made at these temperatures.

Temperature (degrees C)	<i>P. canadensis</i> ¹		<i>P. glaucus</i>		Hybrids		<i>P. troilus</i> ²	
	male	female	male	female	male	female	male	female
30	4.3 ± 0.2	5.9 ± 0.3	—	—	4.8 ± 0.3	(none emerged)	9.2 ± 0.2	11.8 ± 0.3
26	7.1 ± 0.5	8.9 ± 0.4	11.7 ± 0.4	14.2 ± 0.3	9.4 ± 0.3	27.8 ± 0.4	15.8 ± 0.5	15.9 ± 0.6
22	—	—	—	—	—	—	24.7 ± 0.6	25.6 ± 1.2
20	11.0 ± 0.2	14.7 ± 0.4	18.9 ± 0.8	21.0 ± 0.0	13.5 ± 1.0	23.0 ± 2.1	—	—
18	—	—	—	—	—	—	53.4 ± 2.0	54.2 ± 1.6
14*	28.0 ± 1.4	30.7 ± 0.7	55.6 ± 1.4	X (none emerged)	37.0 ± 0.9	X (none emerged)	132.7 ± 4.8	112.6 ± 4.9

¹ *P. canadensis*, *P. glaucus* and hybrid data at 26°, 20°, 14°C are from Keefover and Scriber (unpubl.) also at 18:6 photophase.

² *P. troilus* data at 26°, 22°, 18°, 14°C are from Deering et al. (2001) also at 18:6 photophase.

* No *P. troilus* emergences were observed for 80 d. After 80 d *P. troilus* pupae were moved to room temperature (24–26°C) where they did emerge. While males appear to emerge on the average earlier than female (protandry), this difference is significant (t-test, p = 0.05) only for *P. canadensis* at 20° and 30°C, hybrids at 20° and 26°C, and *P. troilus* at 30°C. Females eclosed significantly later in *P. troilus* at 14°C, but this was after the 80-d experimental period in a warm holding chamber (see Deering et al. 2001).

development and the pupae all obligately diapause. These areas of southeastern and south central USA frequently experience several consecutive days (or weeks) with daily high temperatures above 36°C (see Fig. 1), often with little relief from the heat in the evening. Global warming at regional levels has made it even more common to have several weeks of such extremely high temperatures, as occurred in 1998 and 1999 across the corn belt. Our study shows that even four days at 36°C was lethal to all *P. canadensis* pupae (with or without twice-daily watering). All hybrid male pupae exposed to the same conditions died or eclosed as deformed adults. Female hybrid pupae survived the exposure to high temperatures (36°C) for 30 d and remained alive six months after the experiment, however, only four of the 20 emerged as normal adults.

In this study, due to unavailability, we were unable to evaluate pupal survival of *P. glaucus* to functional (flying) adults. However, a sympatric congener with a geographic range extending from Michigan into southern Florida (*P. troilus*) did do considerably better than the northern swallowtail *P. canadensis* at these higher temperatures. At 30°C, >80% of *P. troilus* pupae produced normal adults (compared to only ca 25% of the *P. canadensis* and 28% of the hybrids; Table 1). However, at 36°C, even individuals of the southern *Papilio* species basically failed to survive and produce normal adults (with the exception of a single male of 36 pupae). It was predicted that southern populations of *P. glaucus* would exhibit similar or better survival of these high temperatures.

Since none of the unclosed *P. canadensis* pupae lived at either 30° or 36°C, it therefore seems likely that after the initial emergence in the first 3–8 d, survival of an extended summer period >30° is not feasible for the Canadian tiger swallowtail butterfly. Consequently, even though obligate diapause (and univoltinism) might theoretically allow a range extension southward for the species, *P. canadensis* would likely not survive as a pure species much to the south of the current hybrid zone even though suitable host plants are abundant all the way to central Florida (Scriber 1988). It remained to be determined if *P. glaucus* would respond differently to 36° and 30°C, reflecting better adaptations to the long hot summer conditions south of the Great Lakes hybrid zone.

However, it was interesting that in our follow-up study (with *P. glaucus* included instead of *P. troilus*), the survival and adult eclosion success of *P. canadensis* and *P. glaucus* were virtually identical, with significant mortality at 36°C (not at 27°, 30°, or 33°C), especially for females (Table 2). Perhaps adaptations associated with the different geographic source of these *P. canadensis* pupae (Bennington Co., Vermont) has resulted in these higher proportions of normal adults at 30°C (90% and 78% for males and females, respectively) than for northern Michigan pupae from our first study

(28% and 20% for males and females, respectively). The higher survival and eclosion success of *P. glaucus* adults from diapausing pupae at 30°C (92–95%; Table 2) may even be better than for *P. troilus* at 30°C (81.4%; Table 1). At 36°C, *P. glaucus* also shows a greater survival and adult eclosion success (35–59%; Table 2) than *P. troilus* (11%; Table 2), however, almost all adults eclosing from pupae at 36°C were deformed in their forewings and/or hindwings. The desiccation and/or heat during the wing expansion may be the mechanism producing deformed adults at 36°C that may not be able to fly.

We do know that *P. canadensis* is behaviorally, morphologically, physiologically, and ecologically better adapted to cold extremes than *P. glaucus* (Kukal et al. 1991, Scriber 1994, Ayres and Scriber 1994). It would seem logical that *P. canadensis* would be less adapted to thermal stress at the high end. At lower temperatures (e.g., 20° and 26°C), pupal survival and adult development are much better for *P. glaucus*, *P. canadensis*, and their hybrids (Keefover and Scriber unpubl.) than we have seen in this study. The same was true for *P. troilus* (Deering et al. 2001). The times to adult eclosion from diapausing pupae were significantly delayed at progressively cooler temperatures for all three species and hybrids (Table 5).

In conclusion, these results show that even a few days at 36°C can be lethal to essentially all diapausing pupae of *P. canadensis* and most of their hybrids with *P. glaucus*. The constraints on southward geographic spread in distribution of *P. canadensis* may largely be determined by these physiological stresses, much like the limited thermal unit accumulations seem to constrain *P. glaucus* gene flow northward (Scriber and Lederhouse 1992, Scriber 1994, Scriber and Gage 1995, Tesar and Scriber 2001). It is interesting that the hybrids may reflect a Haldane effect in heterogametic females (Table 1; Hagen and Scriber 1995) that delays eclosion, but keeps some of the female pupae alive through higher temperatures to eclose the next year (Rockey et al. 1987a, b). Thus increased Haldane effects in the heterogametic sex at higher temperatures (Wade et al. 1999) may not always be bad (Arnold 1997). While deeper diapause of heterogenetic hybrid females of our *P. glaucus* × *P. canadensis* hybrids appears to help survival during higher temperature regimes, it should be noted that they also suffer long delays in eclosion and a reduced likelihood of adult eclosion at a time that would assure presence of males for mating. More research would be needed to determine if this hybrid female benefits in any way from the delayed eclosion and extended pupal diapause, but more hybrid pupae live than either parental species.

The 1999 season was among the hottest ever experienced by the midwestern and eastern USA, with 2–8 consecutive mid-July days exceeding 35°C basically everywhere south of the Great Lakes hybrid zone and at

altitudes lower than the Appalachian Mountains (Fig. 1). This heat extreme continued throughout much of August as well. Whether diapausing pupae of *P. canadensis* may be constrained to their current geographic range north of the hybrid zone for this reason remains uncertain. Our results here suggest that *P. glaucus* would also have a potentially serious problem eclosing successfully from pupae subjected to these high temperatures exceeding 35°C degrees essentially across its entire geographic range east of the Great Plains (Figs 1, 2). It will therefore be interesting to see if serious population declines in *P. glaucus* have occurred.

It is interesting that the 2000 field season was cooler than normal for the state of Michigan and, in sharp contrast to the pattern of hot days in 1999. There were absolutely no days in the entire month of July (2000) above 35°C anywhere north of Tennessee, North Carolina, and Missouri (Fig. 3). This pattern of no days exceeding 35°C also persisted through the first three weeks of August and turned out to be among the coolest July and August months on record.

This highly variable thermal environment illustrates how the "average" pattern of climate may not be the active natural selection pressure. The key factors constraining geographic range distributions for insect herbivores such as these Lepidoptera may occur as an extreme only once a decade, or once every 30–50 yr. For example, a sudden cold year (1992) after several progressively warmer years, extensively froze 3rd, 4th, and 5th instar *Papilio cresphontes* larvae in Michigan (to the extent that it was several years before the species was subsequently observed anywhere in the southern half of the State; Scriber and Gage 1995).

We present our results with the suggestion that unusually "hot" periods, as well as unusually "cold" periods, even if rare, may be devastating to the insect herbivores normally present. These rarely observed climate/weather extremes may be major abiotic factors shaping range distributions of numerous organisms, even though their direct biotic effects are not easily observed.

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