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.

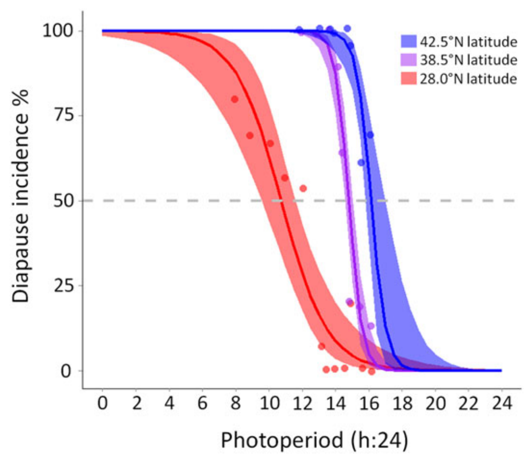

Fig. 1 Photoperiod response curves for the production of diapausing *P. glaucus* pupae at 25 °C from different latitudes across the species' range. The critical day length is the photoperiod at which 50% (dashed gray line) of individuals underwent diapause. The different colors represent the different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