LETTERS

Letter: Ecological mechanism of climate-mediated selection in a rapidly evolving invasive species

Alexandra A. Mushegian,[1]* (iD)
Naresh Neupane,[1] Zachary
Batz,[1] (iD) Motoyoshi Mogi,[2]
Nobuko Tuno,[3] Takako Toma,[4]
Ichiro Miyagi,[4] Leslie Ries[1] and
Peter A. Armbruster[1]

Abstract

Recurring seasonal changes can lead to the evolution of phenological cues. For example, many arthropods undergo photoperiodic diapause, a programmed developmental arrest induced by short autumnal day length. The selective mechanisms that determine the timing of autumnal diapause initiation have not been empirically identified. We quantified latitudinal clines in genetically determined diapause timing of an invasive mosquito, *Aedes albopictus,* on two continents. We show that variation in diapause timing within and between continents is explained by a novel application of a growing degree day (GDD) model that delineates a location-specific deadline after which it is not possible to complete an additional full life cycle. GDD models are widely used to predict spring phenology by modelling growth and development as physiological responses to ambient temperatures. Our results show that the energy accumulation dynamics represented by GDD models have also led to the evolution of an anticipatory life-history cue in autumn.

Keywords

Aedes albopictus, climatic adaptation, diapause.

Ecology Letters (2021)

INTRODUCTION

Understanding how organisms respond to fluctuating environmental conditions is a fundamental goal of biology (Levins 1968; Barton & Turelli, 1989; Lande 2019). Rapidly changing environmental conditions may elicit direct physiological responses at the individual level (phenotypic plasticity) as well as genetic (evolutionary) changes at the population level. Additionally, fluctuating environments can select for genetically based changes in the expression of phenotypically plastic traits, leading to changes in phenotypic reaction norms and the evolution of signals and cues. Understanding these phenomena has particularly important implications for anticipating biological responses to climate change, which is expected to affect many dimensions of environmental suitability and therefore many organismal traits.

In temperate regions, global climate change primarily lengthens the growing season and moderates winter temperatures (Sparks & Menzel 2002; Kunkel 2004). As a result, some of the most prevalent documented responses to climate warming are alterations to the seasonal timing of major life-history events such as emergence, reproduction and entry into winter dormancy (Parmesan 2006; Lavergne et al. 2010). However, most of these responses have been characterised at the phenotypic rather than genetic level (Cohen et al. 2018), so the relative contributions of plastic physiological responses, genetic responses to selection and selection on expression of plasticity are unclear. As a result, it remains difficult to make specific predictions about evolutionary responses to changing seasonal conditions (Varpe 2017).

The recurring environmental extremes of temperate winters represent significant challenges to survival, but are also highly predictable. Accordingly, adaptations that allow organisms to prepare for seasonal environmental changes are expected to be under strong selection (Bradshaw & Holzapfel 2007; Williams et al. 2017; Bacigalupe et al. 2018). In many arthropods, a crucial ecological adaptation is autumnal photoperiodic diapause (Tauber et al. 1986; Danks 1987), an alternative developmental program in which day length provides an anticipatory cue for initiating developmental arrest, increased nutrient storage and stress resistance in advance of unfavourable conditions (Tauber et al. 1986; Denlinger 2002; Koštál 2006). In the Northern Hemisphere, entry into diapause occurs earlier in the year in more northern locations, because winter arrives earlier at northern relative to southern latitudes. This correlation (cline) between latitude and the seasonal timing of diapause initiation is among the most robust biogeographic patterns known, having been documented in hundreds of arthropods (Tauber et al. 1986; Danks 1987; Joschinski & Bonte 2019), and the timing of dormancy has been implicated in rapid adaptation to ongoing climate change in a wide range of taxa (Bradshaw & Holzapfel 2001, 2007; Parmesan 2006; Lavergne et al. 2010).

Photoperiod (day length) provides a precise indicator of the day of the year at a specific latitude. Because photoperiod is an anticipatory cue for diapause, seasonal conditions are

[1]*Department of Biology, Georgetown University, 3700 O St NW, Washington, DC 20057, USA*

[2]*Division of Parasitology, Faculty of Medicine, Saga University, Nabeshima 5-1-1, Saga 849-8501, Japan*

[3]*Laboratory of Ecology, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan*

[4]*University Museum (Fujukan), University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan*

*Correspondence: E-mail: amushegian@gmail.com

Leslie Ries contributed equally to this work with Peter A. Armbruster.

typically still favourable for growth and development when the initiation of diapause occurs, making it challenging to determine the precise environmental factors that are causing selection on the seasonal timing of entry into diapause. A variety of theoretical models have been proposed to explain the optimal timing of entry into diapause (Levins 1968; Cohen 1970; Taylor 1980, 1986a; Hairston, & Munns, 1984). With regard to autumnal diapause, a series of models by Fritz Taylor (Taylor 1980, 1986a,b) have been particularly influential. Taylor's models assume that early entry into diapause incurs a partial loss of fitness due to the physiological cost of diapause or the opportunity cost of ceasing reproduction while conditions are still favourable. Conversely, late entry into diapause incurs mortality and complete loss of fitness for non-diapausing individuals due to catastrophic winter conditions. Within this framework, researchers have often assumed that evolutionary differences in autumnal diapause timing among populations are selected for by differences in the timing of climate events such as the first hard frost (Bradford & Roff 1993; Hard et al. 1993).

However, despite considerable theoretical attention, almost no empirical evidence exists regarding the specific mechanisms of natural selection that affect the seasonal timing of autumnal diapause initiation (Tauber & Tauber 1976; Tauber et al. 1986). It is not obvious what combination of climatic factors best represent the 'arrival of winter' from the point of view of an organism committing to a costly alternative developmental trajectory. For example, a recent meta-analysis found only a weak correlation in Diptera between extrapolated date of diapause entry and winter onset as arbitrarily defined by the fifth day below 10 °C after midsummer (Joschinski & Bonte 2019). Furthermore, our previous work found that relative dates of first hard frost did not correspond to relative diapause timing between two continents in an invasive mosquito (Urbanski et al. 2012). One approach to elucidating the selective mechanisms acting on diapause timing is to identify environmental correlates of population variation. However, all studies of which we are aware that relate diapause clines to environmental correlates are comparisons among populations on a single continent (e.g. Bradshaw et al. 2004; Schmidt et al. 2005; Demont & Blanckenhorn 2008). Because almost all metrics of climate are likely to vary in a consistent and highly correlated way across a single continent, it is difficult or impossible to discern the specific environmental factors that the token cue of photoperiod anticipates, other than 'impending winter' in a general sense.

Here, we provide evidence that across continents, photoperiod predicts the amount of thermal energy for growth remaining in each season. Using replicated common-garden studies of Aedes albopictus collected in 2018 and 2008, we examined genetic differences in diapause timing across latitudes in the mosquito's native range (Japan) and invasive range (North America). Population-level diapause timing exhibited parallel latitudinal clines and consistent latitude-specific differences between the United States and Japan. The latitude-specific differences between the United States and Japan contradict expectations based on defining winter onset by the date of first frost. In contrast, the variation in diapause timing within and between regions is well accounted for by cumulative autumnal temperature conditions quantified using a growing degree-day (GDD) model. GDD models represent the accumulation of thermal energy best suited for growth across the season, calculating how much energy is needed to achieve each developmental milestone. We show that GDD models can provide a useful mechanistic framework to account for the temperature-dependent growth dynamics of the fall seasonal transition, wherein unsuitable winter conditions do not occur abruptly, but are preceded by gradually cooling conditions under which insect development slows down.

GDD models are widely used for predicting the spring phenology of plant and pest species in agriculture (Nietschke et al. 2007) by modelling growth and development as biophysical responses to ambient weather conditions. Our results are the first to show that the energy accumulation dynamics represented by GDD models have led to the evolution of the anticipatory life-history transition of entering diapause in the autumn. By linking local temperature dynamics to a model of insect physiology, our results implicate autumnal GDD as the selective mechanism causing rapid evolution of diapause. This approach connects studies of phenotypic and evolutionary responses and thus may enhance predictions of changes in seasonal timing across geographic and temporal gradients in a wide range of temperate arthropods.

MATERIALS AND METHODS

Study system and experimental design

Aedes albopictus invaded the United States from Japan in 1985, probably as the result of a shipment of used tires (Hawley et al. 1987). In 2008, we used a common garden experiment to compare the diapause incidence and diapause timing (critical photoperiod, CPP: see below) of Ae. albopictus populations between 26 and 41°N in the United States and Japan (Urbanski et al. 2012). In 2018, we repeated the 2008 experiment using the same methods and populations sampled from many of the same locations (Fig. 1, Table S1; complete data in Dryad archive; details in Figs S1-S2, Table S2). In the photoperiodic diapause of Ae. albopictus, the adult female is photosensitive and diapause occurs at the pharate larval stage inside the chorion of the egg (Armbruster 2016). The characteristics measured for each population were as follows: diapause incidence (proportion of eggs that enter diapause) under unambiguous short-day conditions, and critical photoperiod (CPP), defined as the number of hours of light in a day that induces diapause in 50% of a population.

Complete data for the CPP experiment, as well as all additional data files and analysis scripts, can be found in the Dryad data archive at https://doi.org/10.5061/dryad.bk3j9kd9r. Literature references used to estimate GDD requirements for Ae. albopictus can be found in Dataset S1. Additional details on materials and methods can be found in the SI Appendix.

Mosquito population sampling and pre-experiment colony maintenance

Mosquito populations were collected as larvae and pupae in the eastern United States and as eggs, larvae, pupae and
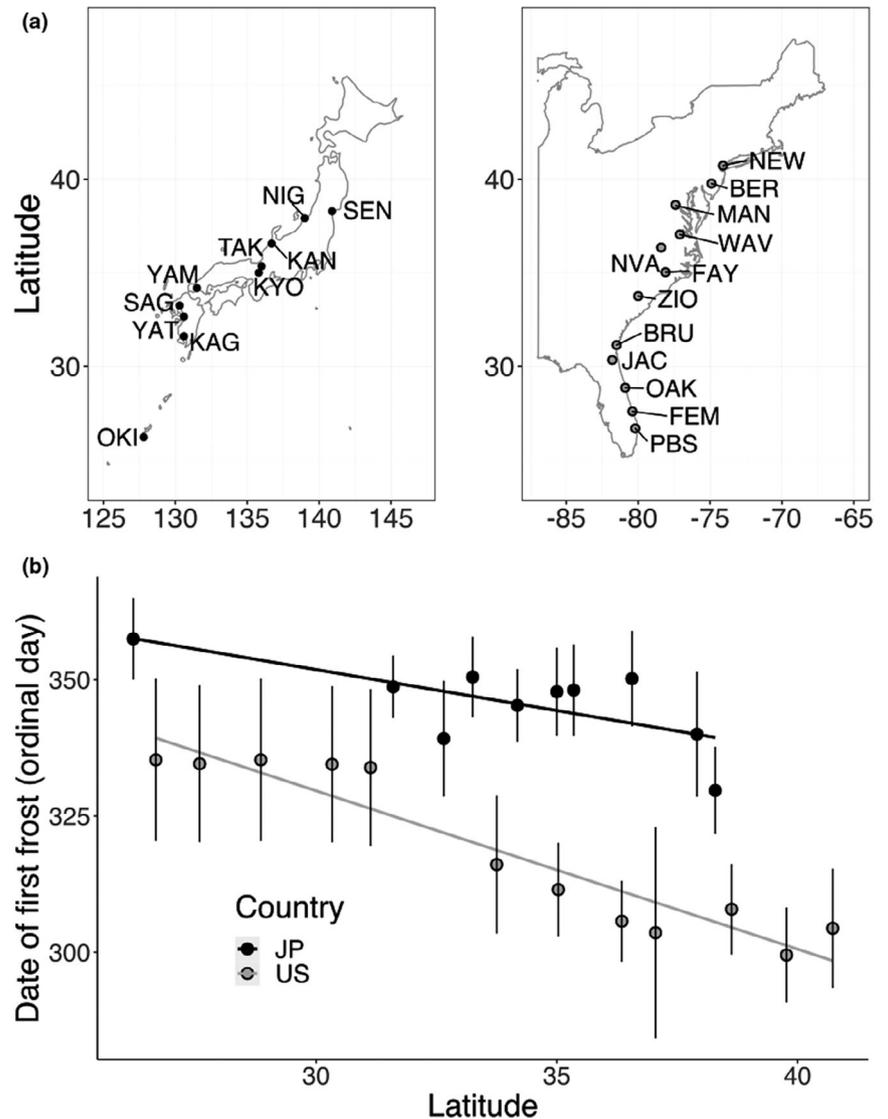
Figure 1 *Aedes albopictus* sampling along a latitudinal gradient in the native and invasive range. Locations in Japan (left) and the United States (right) (a) and mean date of first frost 2008–2017 (b) of sites where mosquitoes were sampled for the 2018 study. Sampling locations in the native (Japan) and introduced (the US) range span a similar range of latitudes. Continent-level climatic differences exist, for example earlier latitude-specific date of first frost in the United States compared to Japan. Error bars represent SD; lines represent linear regression

adults in Japan. All collections occurred between July and September 2018, at latitudes ranging from 26.2 to 40.73°N (Table S1). Locations in the United States were identical to locations sampled in 2008 (Urbanski *et al.* 2012). Japanese population samples were sent to Georgetown University (Washington, DC, USA) as field-collected, F1 or F2 eggs.

Populations were maintained in the laboratory for three or four generations prior to the experiment in a walk-in controlled temperature room at 21°C and 80% relative humidity under an unambiguous long-day (LD) photoperiod (16:8, L: D) as described previously (Armbruster & Conn 2006; Urbanski *et al.* 2012). Larval rearing conditions are described in the SI Appendix. Pupae were then transferred into one adult cage per population. Populations generally had at least 50 female and 50 male adults per generation, but one population (JAC) experienced visibly high mortality during the F2 generation. Adult females were provided a human blood meal 7–14 days post-eclosion. Beginning 4 days post-blood meal, females were provided an oviposition cup lined with an unbleached paper towel and half-filled with deionised water. Eggs were collected Monday-Wednesday-Friday (MWF), maintained on a wet

paper towel for approximately 48 h, then gently air-dried and stored.

Critical photoperiod determination

The experimental design and most of the equipment and experimental details were the same as those used in Urbanski et al. (Urbanski *et al.* 2012); additional details on all steps are provided below and in SI Appendix. An overview of the procedure used to determine the critical photoperiod (CPP) is as follows: Starting from the pupal stage, mosquitoes were divided among 12 different photoperiods ranging from unambiguous short-day (8:16, L:D) to unambiguous long day (16:8, L:D) (listed in SI Appendix) and allowed to produce eggs. The proportion of diapause eggs at each photoperiod was determined, and a dose-response curve was constructed of the proportion of eggs in diapause as a function of photoperiod. The CPP was calculated as the number of hours of light at which 50% of the eggs produced were in diapause, excluding the fraction of constitutively non-diapausing eggs (i.e. the fraction of eggs that did not enter diapause under

unambiguous short-day (SD) conditions (8:16, L:D)). CPP was calculated as the effective dose (ED50) value of a log-logistic dose–response curve of the proportion of diapause eggs as a function of photoperiod.

The experiment was conducted in two blocks, with each block containing half of the populations from each country; populations across the full range of latitudes from each country were interspersed between the blocks. At the beginning of each block, eggs were hatched by submerging oviposition papers in distilled water with larval food slurry for 2 days. Larvae from each population were distributed among 40 15-cm petri dishes containing 90 mL of a mixture of distilled water and larval food at a density of 30-40 larvae per dish; larvae were transferred to clean dishes containing fresh water/food mixture every MWF until pupation (see SI Appendix for more details). Larvae were maintained in a controlled temperature room at 21 °C, 80% relative humidity and LD photoperiod.

Pupae from each population were collected, pooled across dishes, and split amongst thirteen 1.5 L adult cages, corresponding to 12 photoperiod cabinets in the walk-in controlled temperature room and an additional short-day (8:16 L:D) incubator (Precision 815, Thermo Scientific) to provide additional data on short-day diapause incidence. The total number of pupae added to each cage ranged from 52 to 106; pupae were not sexed and subsequent pupal and adult mortality was not monitored. Approximately one week after the last pupae were added, cages were provided with a blood meal, and an oviposition cup half-filled with water and lined with unbleached paper towel was added to each cage. Oviposition papers were collected 5–7 days after the blood meal and replaced every MWF; a total of four oviposition papers were collected from each cage. Several cages were provided a second blood meal and an additional oviposition paper was collected; see SI Appendix for details. Oviposition papers were dried gently and stored in the photoperiod cabinets from which they originated for 11–21 days until quantification of diapause.

To quantify diapause incidence, eggs on each oviposition paper were counted (subsampling if oviposition was very high; see SI Appendix) and papers were submerged in distilled water with larval food slurry. Larvae that hatched were counted 2 days later and papers were re-dried and stored as before. After 1–2 weeks, hatch was induced again and larvae were counted. Oviposition papers were then treated overnight with a bleach solution to clear the chorion of the remaining eggs (Trpis 1970). Eggs were examined under a stereomicroscope, and those eggs containing a pharate larva were scored as embryonated and in diapause. The total number of hatched larvae and embryonated eggs was considered the number of viable eggs. Oviposition papers in which viable eggs made up less than 60% or more than 100% of the eggs initially counted on the paper were considered to be less reliable data points and excluded from further analysis (<10% of papers excluded, see SI Appendix and Fig. S1). Analysis dates, subsampling and other detailed information about each oviposition paper can be found in the file Ae_albopictus_CPP_experiment_data_collated.csv in the Dryad data archive at https://doi.org/10.5061/dryad.bk3j9kd9r.

For each cage, the counts of hatched larvae and embryonated eggs across all oviposition papers were pooled to calculate the fraction of total viable eggs that entered diapause (# embryonated unhatched / (# embryonated unhatched + # hatched)). The median total number of viable eggs per cage was 697 (range 101-1587). For the unambiguous SD photoperiod, each population produced data from two cages as described above. These data were pooled to calculate the value for diapause incidence (DI) (Fig. S3), but used as separate data points in the photoperiod response curves used to calculate CPP (Fig. 2).

Photoperiod response curve data from 2008 were obtained from the data files of Urbanski et al (https://doi.org/10.5061/dryad.68277435) and CPP values were re-calculated to confirm reproducibility of the calculation method.

Ae. albopictus growing degree days

We calculated the estimated number of growing degree days (GDDs) required to complete a full generation and enter diapause, from oviposition of an egg to completion of embryological development of an egg in the next generation (the stage at which diapause occurs). We compiled data from reviews and primary literature that quantified the growth of developmental stages at multiple temperatures (see Dataset S1). We used 10 °C as the baseline temperature required for *Ae. albopictus* growth (Komagata *et al.* 2017). For every temperature/growth trial found in the papers examined, the number of degrees above 10 °C was multiplied by the number of days required to complete each specific life stage to obtain the number of GDD required to reach each developmental milestone. The minimum and maximum values (across studies and tested temperatures) for each life stage were noted, excluding large outliers, and the values for all life stages were summed. The sum of growing degree days for all life stages ranged from 345 to 465, but these values include larval and pupal development rates for male mosquitoes, which develop
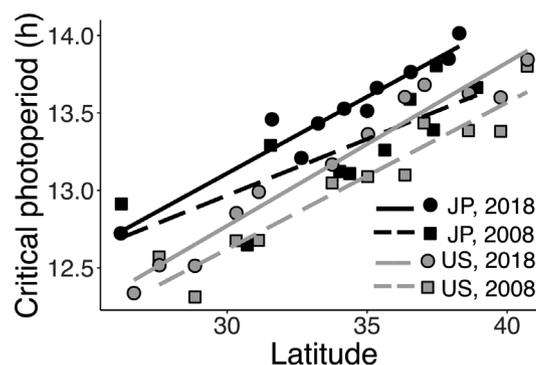

Figure 2 Relationship between diapause timing and latitude in Japan and US, 2008 and 2018. Critical photoperiod strongly correlates with latitude in populations from the United States and Japan in 2008 and 2018, forming parallel clines. Longer latitude-specific critical photoperiods in Japan compared to the United States imply entry into diapause earlier in the season, despite the later date of first frost (Fig. 1b); this difference between clines was present in both 2008 and 2018. Lines represent linear regressions; see Table 1

significantly faster than females; thus, the minimum and maximum GDD for females were taken as 400 and 465 GDD.

Location-specific growing degree day calculations

Daily maximum and minimum temperatures for each sampling location (site) were obtained from the Climate Prediction Center of the National Oceanic and Atmospheric Administration (CPC NOAA, https://www.esrl.noaa.gov/psd/). These observations are available at 0.5° spatial and daily temporal resolution. Data were missing in the CPC dataset for three sites in Japan (Okinawa, Tanegashima, and Shimonoseki); for these locations, we used the European Center's Medium Range Weather Forecasting/ERAI reanalysis (Dee et al. 2011). This reanalysis is available at 0.125° resolution from 1979 to present.

These temperatures were used to calculate daily GDD available at each site, with minimum and maximum thresholds of 10 °C and 33 °C, respectively, as described in Baskerville & Emin (1969). For each year at each site, daily GDD values were accumulated backward beginning from December 31. This determined the GDD-based diapause deadline as the latest day of the year (ordinal day) in which 400 or 465 GDD remained available in the season (GDD-400 or GDD-465).

Additional local climate data

As an additional set of climate variables related to the onset and severity of winter that could conceivably affect diapause timing, the following data were obtained from the climate sources described above for each location for 1998–2017: date of first frost (defined as the first day after midsummer reaching a temperature below 0 °C, or, if there were no frost days in a particular location in a year, the latest first frost day among locations in that country that year); total frost days in the year; minimum, mean, and maximum average daily temperature in the coldest month of each seasonal year (with the coldest month defined as the month with the lowest average monthly temperature); annual temperature variation (defined as the difference in monthly average temperature between the warmest and coldest month) and temperature seasonality (defined as the standard deviation of the monthly average temperatures).

Diapause field data collection

To estimate the seasonal timing of diapause initiation in a natural population at a mid-latitude US site (38°54′N, 77°04′W), we used a previously collected field dataset consisting of eggs (n = 3466) oviposited by wild adult females between July 12 and November 6, 2015. Oviposition traps consisted of 400 mL black cups containing 225 mL deionised tap water (Leisnham & Juliano 2009; Leisnham et al. 2014). Each cup was lined with a piece of non-bleached seed germination paper. Across the period of egg collection, between three and six cups were placed in semi-forested locations on the Georgetown University campus. Papers with eggs were collected from cups and replaced every MWF. Collected papers were transferred to a petri dish and maintained in

sealed plastic containers under field conditions adjacent to the oviposition cup for 2–3 days. Egg papers were then brought into the laboratory for approximately 4–5 hours while eggs were counted and gently dried, then returned in sealed plastic containers to field locations for 12–17 days. Thereafter, eggs were stimulated to hatch twice approximately one week apart and diapause incidence was calculated as for laboratory-based CPP measurements described above. Values from the three collection days each week were combined into a single weekly value and the ordinal day corresponding to the Wednesday of each week was used to construct the photoperiod response curve (Fig. 3a). On 13 out of the 18 weeks of collection, larvae from the first hatching stimulus were raised to pupae using the standard laboratory protocol described above and pupae were identified as Ae. albopictus based on the presence of fringe setae on the anal papillae. Two collection days in the first 3 weeks of collection had low fractions of Ae. albopictus (33% and 58%), but across the entire collection period the median weekly percentage of Ae. albopictus was 99.2%.

Statistical analysis

Critical photoperiod was calculated from photoperiod response curves (Fig. S2) as ED50 using a five-parameter generalised log-logistic model in the R (3.6.0) package drc (3.0.1) (Ritz et al. 2015). Critical photoperiod and diapause incidence data were analysed with linear models and non-parametric tests in base R. Principal component analysis was conducted on additional climate data using the packages stats (3.6.2) and ggbiplot (0.55). Data manipulation and visualisation were carried out using packages in tidyverse (1.2.1) (Wickham et al. 2019), cowplot (1.0.0), ggmap (3.0.0.9), maps (3.3.0) and mapdata (2.3.0).

RESULTS

We calculated CPP of Ae. albopictus populations collected across a similar range of latitudes on two continents (Fig. 1a). Across this range, Japan has a later latitude-specific date of first frost than North America (Fig. 1b). The common garden experiment revealed that CPP correlated with latitude within the United States and Japan in both 2008 and 2018 ($P < 0.001$, Fig. 2, Table 1). The intercept of the latitudinal clines shifted between 2008 and 2018 ($P < 0.001$), but latitudinal clines in 2018 were nearly parallel to those observed in 2008, as verified by the lack of a significant interaction term between year and latitude ($P = 0.17$). In both 2008 and 2018, latitude-specific CPP was higher in Japan than in the United States ($P < 0.001$), implying earlier entry into diapause at the same latitude in Japan than in the United States, in contrast to the expectation based on date of first frost (Fig. 1b).

As an alternative to using date of first frost to delineate the end of the mosquito growing season, we implemented a novel approach using growing degree days (GDDs). Estimates from the literature suggested that it takes 400 to 465 GDDs to complete a mosquito generation, from oviposition through larval, pupal and adult development and the laying and development of a new viable egg. We calculated a hypothetical end of the growing season, or diapause 'deadline', occurring on
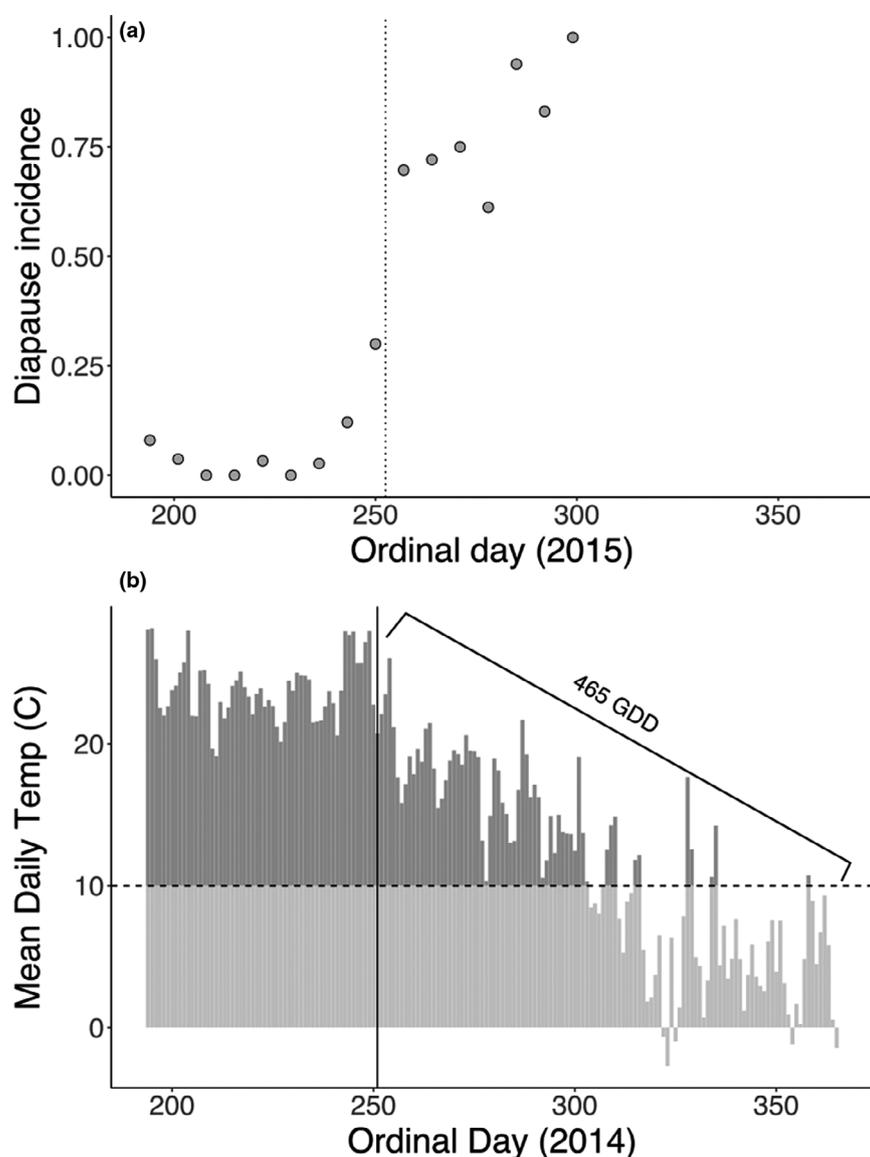
Figure 3 Growing degree day (GDD) deadline corresponds to the seasonal timing of diapause egg production in a natural population. (a) Diapause incidence of eggs produced by a field population of *Aedes albopictus* in Washington, DC, USA during summer and autumn of 2015. The dotted line represents the date at which 50% of eggs are in diapause. (b) Schematic representation of the GDD-465 deadline (vertical line) using temperature time series of summer and autumn 2014 in Manassas, VA, USA, the study site closest (within 50 km) of the DC field site. Daily average temperatures above 10 °C allow mosquito growth and development. Ordinal day 252 represents the GDD-465 deadline in 2014. After this date, there are insufficient GDDs remaining to allow mosquitoes to complete an additional generation; this deadline coincides with the date in 2015 when most mosquito eggs laid were observed to be in diapause

the latest date in the year at a particular location where there are enough GDDs left to complete another generation. We compared this calculated deadline to diapause field data. The field estimate of diapause egg production at a site in Washington, DC indicated that the ED50 occurred on September 7, 2015, corresponding to ordinal day 252.5 (Fig. 3a). In 2014, the calculated deadline for GDD-465 at a nearby site was ordinal day 251 (Fig. 3b). To further examine the performance of the GDD deadline parameter, we calculated deadlines assuming a range of values of GDD required per generation, from 300 to 600. The resulting GDD deadlines were ~4 weeks apart, and the actual date of 50% diapause in the field occurred between 400 and 450 GDD (Fig. S4), within the interval that we predicted based on our review of experimental literature. We proceeded using the 465 GDD deadline for analysis of clinal variation in CPP because this value represents the most conservative estimate for the time required to complete a generation based on the experimental literature.

Daily temperature data were used to calculate a deadline date for every year 1998-2017 at the locations where study populations were sampled. Critical photoperiods measured in 2008 and 2018 correlated with the mean GDD deadlines of the preceding decade (Fig. 4, Table 2) as well as of the

Table 1 Effects of latitude, country and year on critical photoperiod

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Latitude	1	6.270	6.270	268.479	<0.001***
Country	1	0.836	0.836	35.801	<0.001***
Year	1	0.523	0.523	22.405	<0.001***
Latitude:Country	1	0.043	0.043	1.849	0.183
Latitude:Year	1	0.046	0.046	1.955	0.171
Country:Year	1	0.008	0.008	0.354	0.556
Latitude:Country:Year	1	0.010	0.010	0.412	0.525

***$P < 0.001$.

preceding single year (Fig. S6, Table S3). The country effect was not significant, indicating that locally calculated GDD deadlines predicted critical photoperiods regardless of country (Table 2). There was a significant GDD deadline-by-country interaction effect in the model in which the 2017 deadline was used as an explanatory variable for 2018 CPP data (Fig. S6b, Table S3b), but not when the GDD deadline was averaged over the preceding 10 years, suggesting that year-to-year variation may additionally affect the relationship between local GDD conditions and local CPP.

DISCUSSION

Cross-continental comparison reveals reproducible differences in genetically determined seasonal timing of diapause initiation

In both 2008 and 2018, under common-garden conditions, Japanese populations of Ae. albopictus entered diapause at longer critical photoperiods, corresponding to dates earlier in the season, the US populations from the same latitude. This contradicts the hypothesis that the date of first frost is the selective factor mediating the timing of entry into diapause on each continent because the date of first frost is later in Japan the at the same latitude in the United States (Fig. 1b). Because the longer latitude-specific CPP in Japan relative to the United States persisted between 2008 and 2018, providing strong evidence for consistent genetically based differences between the continents, this discrepancy indicates that another selective mechanism must be causing the latitude-specific difference in the timing of diapause initiation between continents.

Growing degree-day models can be used to define the autumnal end of the growing season

We implemented a novel approach to modelling the optimal date for entering diapause and corroborated it using field data from a mid-latitude US location. We reasoned that the growing season would be limited based on the amount of time required to complete a full generation under particular climatic conditions. Insect phenology studies often estimate generation times using growing degree-day (GDD) models based on the observation that insect development rate is related linearly to temperature between approximately 10 and 30 °C (Rebaudo & Rabhi 2018). GDD models represent development as an accumulation of thermal degrees in this growing range. Species-specific temperature-dependent growth rates, while individually variable, tend to show little geographic variation (Buckley et al. 2017), and previous studies showed similar temperature-response growth curves between genetically distinct Ae. albopictus populations (Kamimura et al. 2002; Healy et al. 2019). Based on our survey of the Ae. albopictus literature, a full generation from oviposition to development of a pharate larva in an egg requires 400–465 GDD. Field data from Washington DC in 2015 showed that the observed date on which 50% of Aedes albopictus eggs produced are in diapause closely corresponds to the date from the previous year at which there were 465 growing degree days remaining in the year (Fig. 3). Unlike CPP data, which
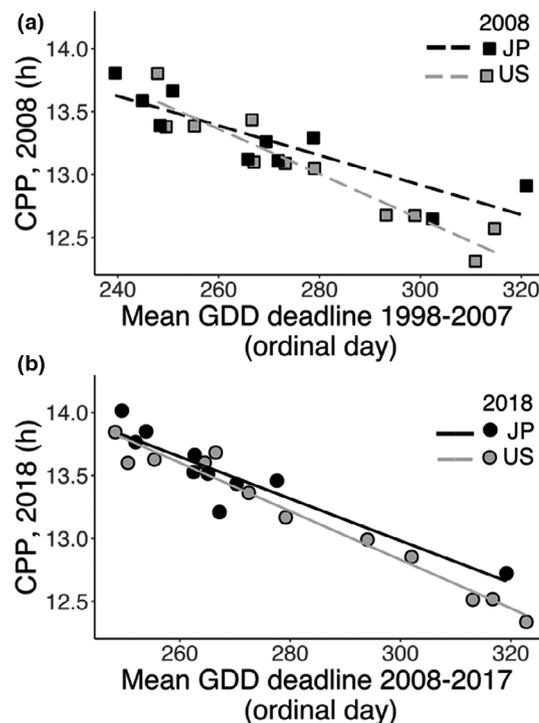
Figure 4 GDD deadline explains CPP across continents. Critical photoperiod measured in the lab for mosquitoes collected in 2008 (a) and 2018 (b) at multiple sites (each point on the graph). The measured CPP for each population is compared to the mean GDD-465 deadline calculated from climate data and expressed as an ordinal day of the year after which individuals must lay diapause eggs to avoid early termination of development and loss of fitness. GDD values are the average across years in the preceding decade for each time period. A similar correlation is found with GDD-465 deadline in the previous single year (Fig. S6; Table S3). Timing of diapause entry as represented by critical photoperiod thus correlates with the date in each location when insufficient growing degree days remain to complete another generation in the season, regardless of country of population origin. These results show that differences in latitude-specific GDD-465 between countries account for differences in latitude-specific CPP between countries. Lines represent results of linear regression; see Table 2 for test statistics

Table 2 Effects of calculated GDD-465 deadlines (mean over previous decade) on critical photoperiods measured in 2008 and 2018

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
(A) 2008 CPP vs. 1998-2007 GDD deadline					
Mean GDD-465	1	2.762	2.762	99.462	<0.001***
Country	1	0.061	0.061	2.212	0.155
Mean GDD-465: Country	1	0.109	0.109	3.924	0.064.
(B) 2018 CPP vs. 2008-2017 GDD deadline					
Mean GDD-465	1	4.494	4.494	287.257	<0.001***
Country	1	0.032	0.032	2.026	0.172
Mean GDD-465: Country	1	0.016	0.016	1.032	0.323

***$P < 0.001$.

serve as a laboratory-based proxy for seasonal timing, the field study directly observes actual dates of diapause entry, so the correspondence between the date of diapause entry and

the calculated deadline date validates this approach. As further validation, we estimated GDD deadlines for 2009 at a site in Mediterranean France, where in 2010 *Aedes albopictus* were found to have initiated diapause in 50% of eggs during the second week of September (September 11). The 400- and 450-GDD deadlines in 2009 at this site fell on September 13 and September 8 respectively. This remarkably close correspondence further supports our novel GDD-based generation time model as a robust predictor of autumnal diapause timing.

Deadlines based on local growing degree days select for differences in diapause timing between continents

We used daily climate data from the past 20 years from our sampling locations in the United States and Japan to calculate a yearly GDD-based 'deadline' for diapause at each location. Yearly GDD deadlines calculated using historical climate data (1998–2017) correlated with latitude, with parallel clines in the United States and Japan, and the latitude-specific GDD deadline was earlier in Japan than in the United States for most years examined (Fig. S5a). This illustrates that different latitudinally varying metrics of the seasonal timing of the onset of winter are not equivalent across continents: although the Japanese locations generally experienced a later date of first frost than US populations at the equivalent latitude (Fig. 1b), the GDD-deadline model based on the distribution of temperature conditions over the autumn season (Fig. S5b) implies an earlier end date for suitable growing conditions in Japan.

The differences in the latitude-specific GDD deadline between Japan and the United States resolve the apparent anomaly of longer latitude-specific critical photoperiod in Japan (Figs 2 and 4). Taken together with the close correspondence between the GDD-based deadline and diapause initiation within a natural population (Fig. 3), these results support the GDD-deadline as the seasonal factor that selects for longer latitude-specific CPPs in Japan relative to the United States. Comparing critical photoperiods of coastal and continental populations would further validate whether temperature-dependent growth rates underlie local and regional variation in diapause timing.

We also observed an overall shift to earlier critical photoperiod between the 2008 and 2018 experiments (Fig. 2, Table 1) which was not explained by GDD-based deadline dates. Although deadlines vary from year to year, there is no discernible directional trend over the 20-year period examined (Fig. S7a). There is also no clear temporal shift across this period in a suite of bioclimatic variables related to the onset and severity of winter (Fig. S7b). Although the experiments in 2008 and 2018 used the same equipment, the same insect husbandry methods, and the same procedures for measuring CPP, we cannot rule out the possibility that slight differences between experiments performed 10 years apart explain at least part of the temporal shift in CPP (Table S2). However, diapause incidence under short-day conditions did not change between 2008 and 2018 (Fig. S3); because this measurement requires fewer data points than calculating CPP, it might be less sensitive to unknown experimental variation and thus may be a better indicator of temporal shifts or lack thereof.

GDD deadline explains rapid evolution of CPP during invasion and range expansion

Our results provide robust evidence of rapid adaptive changes in CPP in response to differences in GDD-based diapause deadline between the native (Japan) and invasive (US) range of *Ae. albopictus*. Because we measured the CPP of all populations under common-garden conditions, the differences we detected represent genetic changes in the interpretation of and response to photoperiod. After a period of rapid evolution in the relative slopes of the clines from 1988 to 2008 (Urbanski *et al.* 2012), the parallel slopes and differences in intercept of the CPP clines in the United States and Japan remained consistent between 2008 and 2018 (Fig. 2, Table 1). The differences in latitude-specific CPPs established in 2008 correlate with GDD-based deadline differences between continents, implying the adaptive evolution of CPP to climatic differences between the United States and Japan had already occurred across the US range of *Ae. albopictus* in 2008 (~70–105 generations). Population genetic studies indicate consistently low levels of population structure at neutral markers among US populations (Medley *et al.* 2015; Kotsakiozi *et al.* 2017; Batz *et al.* 2020), further supporting the conclusion of strong selection driving rapid differentiation along this latitudinal gradient.

While the proximal cues that regulate diapause initiation, such as photoperiod and temperature, have been extensively studied and are relatively well understood, almost no direct evidence exists concerning the specific environmental gradients that are mechanistically relevant to establishing genetic clines (Tauber *et al.* 1986). Our results provide evidence for a specific driver linked to a cue; specifically, cumulative temperatures across late summer and early fall are an important cause of selection affecting the evolution of the seasonal timing of entry into diapause. A combination of sensitive sensory mechanisms for matching life history transitions to time of year and the drastic fitness consequences of mistiming diapause entry appears to have resulted in remarkably precise adaptation of diapause timing to local growing season length across a global geographical range in a relatively short period of time. Under this model, photoperiod is a token cue that accurately predicts reduced future opportunity for growth due to declining temperatures and thus initiates entry into diapause despite ambient conditions that are still favourable for growth and development. Selection, therefore, results from fitness loss due to seasonally declining temperatures and the limitations imposed by the energetic requirements of development.

These results suggest that studies of a wide range of evolutionary and ecological dynamics under altered seasonality caused by global climate change will benefit from integrating ecophysiological approaches such as GDD models. Because temperature has a profound impact on biochemical processes in all ectothermic organisms, it will be interesting to explore whether the autumnal GDD-deadline approach we have developed is effective for predicting evolutionary changes in the initiation of diapause in other organisms and subsequent adaptation to changing environments.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

PAA conceived the experiment and collected mosquitoes in North America. AAM carried out the experiment and analysed data. ZB contributed to carrying out the experiment and collected field data in Washington DC. LR and NN developed the GDD model and NN compiled and analysed climate data. MM, NT, TT and IM collected mosquitoes in Japan. AAM and PAA wrote the paper. All authors revised and commented on the paper.

DATA AVAILABILITY STATEMENT

Data and code used for analysis are archived in Dryad at https://doi.org/10.5061/dryad.bk3j9kd9r.

REFERENCES

Armbruster, P.A. (2016). Photoperiodic diapause and the establishment of *Aedes albopictus* (Diptera: Culicidae) in North America. *J. Med. Entomol.*, 53, 1013–1023.

Armbruster, P. & Conn, J.E. (2006). Geographic variation of larval growth in North American *Aedes albopictus* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, 99, 1234–1243.

Bacigalupe, L.D., Gaitán-Espitia, J.D., Barria, A.M., Gonzalez-Mendez, A., Ruiz-Aravena, M., Trinder, M. et al. (2018). Natural selection on plasticity of thermal traits in a highly seasonal environment. *Evol. Appl.*, 11, 2004–2013.

Barton, N. & Turelli, M. (1989). Evolutionary quantitative genetics: how little do we know? *Annu. Rev. Genet.*, 23(1), 337–370.

Baskerville, G.L. & Emin, P. (1969). Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology*, 50, 514–517.

Batz, Z.A., Clemento, A.J., Fritzenwanker, J., Ring, T.J., Garza, J.C. & Armbruster, P.A. (2020). Rapid adaptive evolution of the diapause program during range expansion of an invasive mosquito. *Evolution*, 74, 1451–1465.

Bradford, M.J. & Roff, D.A. (1993). Bet hedging and the diapause strategies of the cricket *Allonemobius fasciatus*. *Ecology*, 74, 1129–1135.

Bradshaw, W.E. & Holzapfel, C.M. (2001). Genetic shift in photoperiodic response correlated with global warming. *Proc. Natl Acad. Sci.*, 98, 14509–14511.

Bradshaw, W.E. & Holzapfel, C.M. (2007). Evolution of animal photoperiodism. *Annu. Rev. Ecol. Evol. Syst.*, 38, 1–25.

Bradshaw, W.E., Zani, P.A. & Holzapfel, C.M. (2004). Adaptation to temperate climates. *Evolution*, 2004, 15.

Buckley, L.B., Arakaki, A.J., Cannistra, A.F., Kharouba, H.M. & Kingsolver, J.G. (2017). Insect development, thermal plasticity and fitness implications in changing, seasonal environments. *Integr. Comp. Biol.*, 57, 988–998.

Cohen, D. (1970). A theoretical model for the optimal timing of diapause. *Am. Nat.*, 104, 389–400.

Cohen, J.M., Lajeunesse, M.J. & Rohr, J.R. (2018). A global synthesis of animal phenological responses to climate change. *Nat. Clim. Chang.*, 8, 224–228.

Danks, H.V. (1987). *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada monograph series. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, Canada.

Dee, D.P., Uppala, S.M., Simmons, A.J., Berrisford, P., Poli, P., Kobayashi, S. et al. (2011). The ERA-Interim reanalysis: configuration and performance of the data assimilation system. *Quarterly Journal of the Royal Meteorological Society*, 137, 553–597.

Demont, M. & Blanckenhorn, W.U. (2008). Genetic differentiation in diapause response along a latitudinal cline in European yellow dung fly populations. *Ecol. Entomol.*, 33, 197–201.

Denlinger, D.L. (2002). Regulation of diapause. *Annu. Rev. Entomol.*, 47, 93–122.

Hairston, N.G. & Munns, W.R. (1984). The timing of copepod diapause as an evolutionarily stable strategy. *Am. Nat.*, 123, 733–751.

Hard, J.J., Bradshaw, W.E. & Holzapfel, C.M. (1993). Genetic coordination of demography and phenology in the pitcher-plant mosquito, *Wyeomyia smithii*. *J. Evol. Biol.*, 6, 707–723.

Hawley, W., Reiter, P., Copeland, R., Pumpuni, C. & Craig, G. (1987). *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science*, 236, 1114–1116.

Healy, K.B., Dugas, E. & Fonseca, D.M. (2019). Development of a degree-day model to predict egg hatch of *Aedes albopictus*. *J. Am. Mosquito Control Assoc.*, 35, 249–257.

Joschinski, J. & Bonte, D. (2019). Diapause is not selected as a bet-hedging strategy in insects: a meta-analysis of reaction norm shapes (preprint). *Ecology*. https://www.biorxiv.org/content/10.1101/752881v4

Kamimura, K., Matsuse, I.T., Takahashi, H., Komukai, J., Fukuda, T., Suzuki, K. et al. (2002). Effect of temperature on the development of Aedes aegypti and Aedes albopictus. *Med. Entomol. Zool*, 53, 53–58.

Komagata, O., Higa, Y., Muto, A., Hirabayashi, K., Yoshida, M., Sato, T. et al. (2017). Predicting the start of the *Aedes albopictus* (Diptera: Culicidae) female adult biting season using the spring temperature in Japan. *J. Med. Entomol.*, 54, 1519–1524.

Koštál, V. (2006). Eco-physiological phases of insect diapause. *J. Insect Physiol.*, 52, 113–127.

Kotsakiozi, P., Richardson, J.B., Pichler, V., Favia, G., Martins, A.J., Urbanelli, S. et al. (2017). Population genomics of the Asian tiger mosquito, *Aedes albopictus* : insights into the recent worldwide invasion. *Ecol. Evol.*, 7, 10143–10157.

Kunkel, K.E. (2004). Temporal variations in frost-free season in the United States: 1895–2000. *Geophys. Res. Lett.*, 31(3), 1895–2000.

Lande, R. (2019). Developmental integration and evolution of labile plasticity in a complex quantitative character in a multiperiodic environment. *Proc. Natl Acad. Sci.*, 116, 11361–11369.

Lavergne, S., Mouquet, N., Thuiller, W. & Ronce, O. (2010). Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annu. Rev. Ecol. Evol. Syst.*, 41, 321–350.

Leisnham, P.T. & Juliano, S.A. (2009). Spatial and temporal patterns of coexistence between competing Aedes mosquitoes in urban Florida. *Oecologia*, 160, 343–352.

Leisnham, P.T., LaDeau, S.L. & Juliano, S.A. (2014). Spatial and temporal habitat segregation of mosquitoes in urban Florida. *PLoS One*, 9, e91655.

Levins, R. (1968). *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press, Princeton, NJ.

Medley, K.A., Jenkins, D.G. & Hoffman, E.A. (2015). Human-aided and natural dispersal drive gene flow across the range of an invasive mosquito. *Mol. Ecol*, 24, 284–295.

Nietschke, B.S., Magarey, R.D., Borchert, D.M., Calvin, D.D. & Jones, E. (2007). A developmental database to support insect phenology models. *Crop Prot.*, 26, 1444–1448.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.*, 37, 637–669.

Rebaudo, F. & Rabhi, V.-B. (2018). Modeling temperature-dependent development rate and phenology in insects: review of major developments, challenges, and future directions. *Entomol. Exp. Appl.*, 166, 607–617.

Ritz, C., Baty, F., Streibig, J.C. & Gerhard, D. (2015). Dose-response analysis using R. *PLoS One*, 10, e0146021.

Schmidt, P.S., Matzkin, L., Ippolito, M. & Eanes, W.F. (2005). Geographic variation in diapause incidence, life-history traits, and climatic adaptation in drosophila melanogaster. *Evolution*, 59, 1721–1732.

Sparks, T.H. & Menzel, A. (2002). Observed changes in seasons: an overview. *Int. J. Climatol.*, 22, 1715–1725.

Tauber, M.J. & Tauber, C.A. (1976). Insect seasonality: diapause maintenance, termination, and postdiapause development. *Annu. Rev. Entomol.*, 21, 81–107.

Tauber, M.J., Tauber, C.A. & Masaki, S. (1986). *Seasonal Adaptations of Insects*. Oxford University Press, Oxford, UK.

Taylor, F. (1980). Optimal switching to diapause in relation to the onset of winter. *Theor. Popul. Biol.*, 18, 125–133.

Taylor, F. (1986a). The fitness functions associated with diapause induction in arthropods I. The effects of age structure. *Theor. Popul. Biol.*, 30, 76–92.

Taylor, F. (1986b). The fitness functions associated with diapause induction in arthropods II. The effects of fecundity and survivorship on the optimum. *Theor. Popul. Biol.*, 30, 93–110.

Trpis, M. (1970). A new bleaching and decalcifying method for general use in zoology. *Can. J. Zool.*, 48(4), 892–893.

Urbanski, J., Mogi, M., O'Donnell, D., DeCotiis, M., Toma, T. & Armbruster, P. (2012). Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. *Am. Nat.*, 179, 490–500.

Varpe, Ø. (2017). Life history adaptations to seasonality. *Integr. Comp. Biol.*, 57, 943–960.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R. et al. (2019). Welcome to the tidyverse. *J. Open Source Software*, 4, 1686.

Williams, C.M., Ragland, G.J., Betini, G., Buckley, L.B., Cheviron, Z.A., Donohue, K. et al. (2017). Understanding evolutionary impacts of seasonality: an introduction to the symposium. *Integr. Comp. Biol.*, 57, 921–933.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.