Inclusion of host quality data improves predictions of herbivore phenology

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Abstract

Understanding the correspondence between ambient temperature and insect development is necessary to forecast insect phenology under novel environments. In the face of climate change, both conservation and pest control efforts require accurate phenological predictions. Here, we compare a suite of degree-day models to assess their ability to predict the phenology of a common, oligophagous butterfly, the silver-spotted skipper, *Epargyreus clarus* (Cramer) (Lepidoptera: Hesperiidae). To estimate model parameters, we used development time of eggs and larvae reared in the laboratory at six constant temperatures ranging from 8 to 38 °C and on two host plants of contrasting quality (kudzu and wisteria). We employed three approaches to determine the base temperature to calculate degree days: linear regression, modified reduced major axis regression, and application of a generic base temperature value of 10 °C, which is commonly used in the absence of laboratory data. To calculate the number of degree days required to complete a developmental stage, we used data from caterpillars feeding on high- and low-quality hosts, both in the field and in the laboratory. To test model accuracy, we predicted development time of seven generations of larvae reared in the field on the same host plants across 3 years (2014–2016). To compare performance among models, we regressed predicted vs. observed development time, and found that r^2 values were significantly larger when accounting for host plant quality. The accuracy of development time predictions varied across the season, with estimates of the first two generations being more accurate than estimates of the third generation, when ambient temperatures dropped outside the range in which development rate and temperature have a linear relationship. Overall, we show that accounting for variation in host plant quality when calculating development time in the field is more important than the choice of the base temperature for calculating degree days.

Introduction

Developing a predictive framework for how environmental variation drives phenological responses is an important goal in insect conservation and pest management. Here, we evaluate the performance of several models to predict the phenology of a common butterfly. Lepidopteran species are particularly sensitive to phenological alterations triggered by climate change (Ellis et al., 1997; Parmesan et al., 1999; Roy & Sparks, 2000; Forister & Shapiro, 2003; Altermatt, 2012; Diamond et al., 2014). Documented phenological responses of lepidopterans inhabiting temperate environments include both increased voltinism (the number of generations a population has in a given year) and altered time of first flight (the period when adults are active for the first time in a growing season). Although some species delay their time of first flight under warmer conditions, the most common trend across taxa, including Lepidoptera, is toward phenological advances (Parmesan, 2007; Diamond et al., 2014). These shifts can be dramatic,

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as in the case of the red admiral butterfly, *Vanessa atalanta* (L.), which advanced its flying time by about a month in North America over a 30-year period (1972–2002; Forister & Shapiro, 2003) and in Europe over a 22-year interval (1976–1998; Roy & Sparks, 2000). Such phenological advances may result in asynchrony between food plants and predators, altering the outcome of trophic interactions (Bale et al., 2002). In temperate ecosystems, earlier flying times also result in extended growing seasons, which can permit multivoltine species to include more generations per year (Altermatt, 2010).

Increases in voltinism can result in insect outbreaks (Steinbauer et al., 2004), exacerbating the pressures herbivores exert on food plants, which is of special concern in agricultural systems (Martín-Vertedor et al., 2010). However, increased voltinism can also trigger population declines when it results in the development of partial generations, wherein a proportion of individuals is unable to reach the overwintering stage prior to the season's end (Van Dyck et al., 2015). Understanding the relationship between lepidopteran phenology and environmental conditions is therefore important for the development of effective conservation as well as pest management strategies.

In the absence of behavioral thermoregulation, the development rate of ectotherms increases linearly with temperature within a given range (Jarošík et al., 2004). Therefore, there is a well-established practice of predicting ectotherm phenology as a function of heat accumulation (Bonhomme, 2000; see Ikemoto & Takai, 2000, for an in-depth explanation). The most common approach is to calculate degree days, which measure the number of degrees Celsius that accumulate over time within a temperature range. Under this approach, phenological events can be predicted using two parameters: t, which is the base temperature used to calculate degree days, and k, which is the number of degree days required for the occurrence of a given phenological phase (phenophase).

Three methods are commonly used to estimate t for insect development. The most popular model (classic model hereafter) estimates t as the x-intercept of a linear regression between temperature and development rate of individuals reared in the laboratory at a range of constant temperatures (Figure 1; see Jarošík & Honěk, 2011 for a compilation of studies). An important and often overlooked step of this process is to identify the temperature interval where developmental rate increases linearly with temperature, discarding any data points that lie outside it. This valid interval is delimited by a lower critical temperature and an upper critical temperature. To better estimate this interval and to minimize other biases inherent in linear regression, Ikemoto & Takai (2000) proposed using

major axis regression (I&T model hereafter) instead of the classic ordinary least squares linear regression. Using this methodology, t is estimated as the slope of the regression between temperature*development time and development time (Figure 1). A third approach is to arbitrarily assign t the value of 10 °C (generic value hereafter) and calculate degree-day accumulation (k) in the field. The rationale for using this much simpler approach is that estimating t is labor-intensive, and most of the t values calculated to date fall around a value of 10 °C, so this is a reasonable approximation (Jarošík & Honěk, 2011). Furthermore, whereas t has been assumed to be constant across ontogeny for a given population (Jarošík et al., 2002), thermal resilience can vary significantly among life stages (Bowler & Terblanche, 2008), resulting in stage-specific t values. In this study, we use development rate of both eggs and larvae to estimate t, following each of the three approaches described above.

Development time of insects reared at a constant temperature may differ from that of insects reared under a fluctuating regime with the same mean (Bryant et al., 1999). This known limitation of degree-day models can be circumvented by calculating k under field conditions and using those values to predict phenology in subsequent years (e.g., Dearborn & Westwood, 2014; Cayton et al., 2015). This practice may also better capture local weather variation, increasing model accuracy for the area where it was developed, but potentially limiting its general applicability. This limitation is less severe when developing pest control models, as multiple models for specific localities are commonly available for major pests; however, when making developmental predictions for novel (or changing) environments, or those that cover large portion of a species' range, the blanket use of locally derived parameters may limit their predictive ability. Here we compare accuracy of predictions when using laboratory- vs. field-calculated k values.

Host plant quality is another factor that complicates phenological predictions, especially for non-pest species, because it substantially affects insect growth rates (Stamp, 1993; Coley et al., 2006), with larvae growing significantly faster when feeding on higher-quality hosts (Segarra-Carmona & Barbosa, 1983; Stamp, 1993; Honěk et al., 2002; Liu et al., 2004; Mason et al., 2011). Despite the well-documented effect of host quality on herbivore development time (Teder et al., 2014), host plants have not been explicitly incorporated into models predicting the phenology of non-pest species. Here, we test whether incorporating host quality data into the various degree-day modeling approaches described above results in better predictions of the phenology of an oligophagous butterfly, the silver-spotted skipper, *Epargyreus clarus* (Cramer) (Lepidoptera:
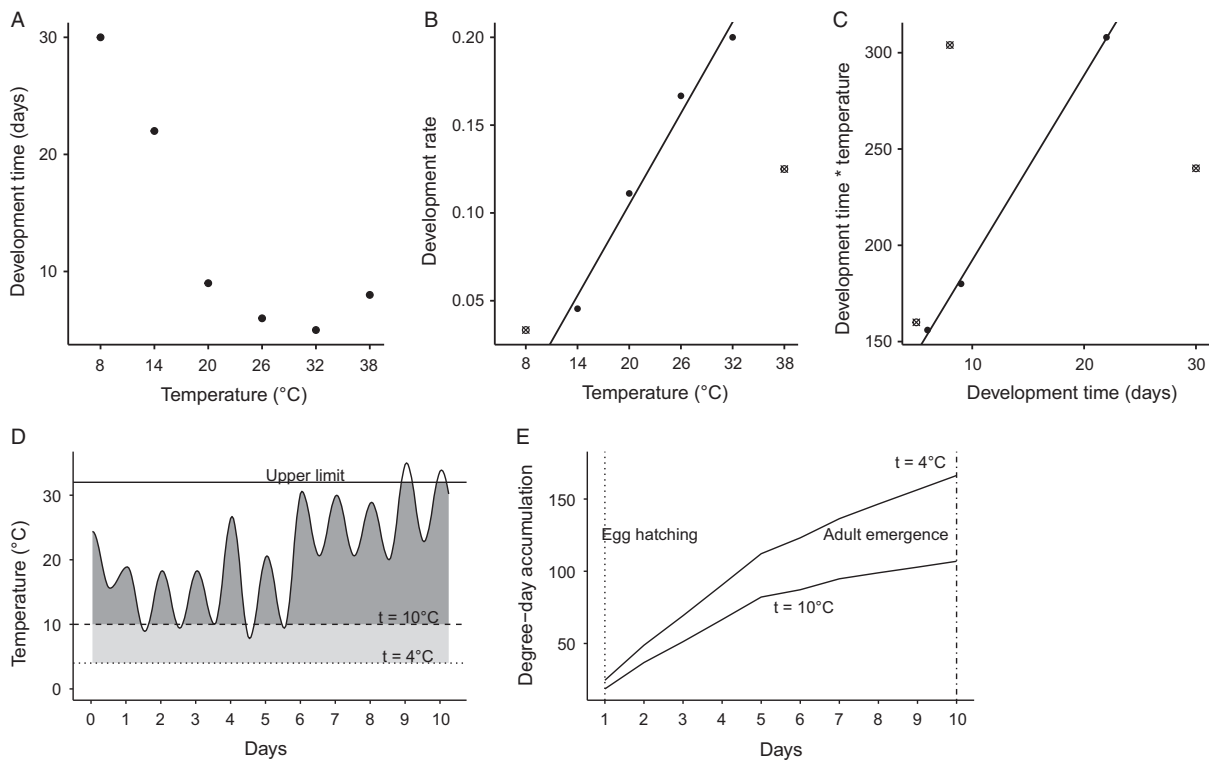
Figure 1 Example of k and t estimation. The top panels illustrate t estimation using (A) hypothetical development time data, to calculate (B) development rate to apply a classic linear regression, and (C) development time*temperature to apply a major axis regression (I&T model). Crossed circles represent data points excluded from the regressions because they fall outside the valid interval, at which development and temperature have a linear relationship. In the case of the classic model, t is the x-intercept, whereas in the I&T model t is the slope of the line. The bottom panels illustrate k calculation assuming two values of t (4 and 10 °C): (D) daily temperature fluctuations, shaded areas represent degree days accumulated assuming t of 4 °C (light + dark gray), and t of 10 °C (dark gray), and (E) degree days accumulated over time from egg hatching to adult emergence of a hypothetical insect.

Hesperiidae). To test model accuracy, we compared predicted to observed development times for seven generations of caterpillars reared in the field on low- and high-quality hosts over a period of 3 years. In addition, we predicted the number of generations *E. clarus* ought to be able to complete in our region when feeding on each of the two host plants.

Materials and methods

Study system

Epargyreus clarus is a multivoltine butterfly native to North America. Its range includes the continental USA and southern Canada, where it typically exhibits 2–3 generations per year. Caterpillars feed on the foliage of several species in the family Fabaceae (Wagner, 2005), where they build characteristic leaf shelters. Each caterpillar inhabits a shelter until it outgrows it, building a total of four or five throughout their lifetime (Weiss et al., 2003). Rosenwald

et al. (2017) assessed the performance of *E. clarus* larvae reared on several commonly used hosts in the Eastern USA. They found that the invasive kudzu, *Pueraria montana* (Lour.) Merr. (Fabaceae), produces leaves with higher nitrogen (ca. 4%) and water (75%) than another invasive legume, wisteria [*Wisteria sinensis* (Sims) Sweet, Fabaceae; ca. 2.5% N, ca. 57% water]. These differences in foliage quality affected the development time and pupal mass of *E. clarus*, with larvae feeding on kudzu developing 33% faster and into significantly larger pupae than those feeding on wisteria (Rosenwald et al., 2017). Because these hosts coexist, are consumed by wild larvae, and encompass the range of variation in quality that larvae are likely to encounter in nature, we selected them to feed our experimental caterpillars.

Laboratory rearing conditions

To determine the temperature range suitable for *E. clarus* egg development, we subjected 20 recently laid eggs (less

than 6 h old) to each of six constant temperature regimes (8, 14, 20, 26, 32, 38 °C) and L16:D8 photoperiod using Percival growth chambers models 136 VL and 130 VL (Percival Scientific, Perry, IA, USA). Eggs were laid over several days by a mix of wild-caught and laboratory-reared females (n = 15 females). We monitored eggs daily, recording mortality and time to larval eclosion.

Similarly, to determine the temperature range suitable for larval development on two host plants of contrasting quality, we assigned hatchlings feeding on kudzu (high quality) or wisteria (low quality) to the same six constant temperature regimes (8–38 °C, L16:D8 photoperiod) until adult emergence or pupal diapause induction. We obtained all larvae (n = 480; 40 in each of 6 temperature*2 host plant combinations) by keeping a combination of wild-caught (Washington, DC, USA) and colony-reared butterflies (founders from both Washington, DC, and nearby areas of Maryland) in an outdoor insectary, provided with nectar plants and kudzu leaves for oviposition. We checked the insectary for eggs daily and we transferred them to a growth chamber at 26 °C and L16:D8 photoperiod (for larval trials only). Upon hatching, we assigned larvae to their respective host plant*temperature treatments and thereafter monitored them daily, recording life stage and mortality, and providing fresh foliage ad libitum. Larvae were kept in individual containers with foliage and a piece of moist filter paper; relative humidity levels ranged from 80 to 90%. We allowed larvae to remain in their shelters when replenishing foliage. We also noted any incidence of supernumerary instars, a common response of larvae growing under thermal stress (temperatures either too low or too high; Esperk et al., 2007). For example, a species that typically develops through five instars may exhibit one or two extra instars when experiencing stressful conditions. We compared larval survival as well as incidence of a sixth instar between hosts and among temperature treatments using χ^2 tests.

Field larvae

To determine larval development time in the field, we maintained a common garden at Patuxent Wildlife Research Center, MD, USA (39.05N, 76.82W) where we grew both wisteria and kudzu plants. We set out an average of 43 hatchlings (1-day-old caterpillars) on each host plant at three times during the growing season: early June, mid-July, and early September of 2014, 2015, and 2016 (total n = 681; Figure 2). We recorded the development stage (instar) of field-reared larvae twice per week and collected them before pupation. We compared development time among generations and years using a generalized linear model assuming a Poisson distribution and with a log link function, including development time as the dependent variable and year, generation, and their interaction as predictor variables. We used the first generation of field caterpillars from 2014 to estimate k values under field conditions on each host plant, and the rest of the generations to test model predictions. We do not report data for the third generation of 2014 because they died prematurely due to an early frost.

Experimental estimation of parameters t and k

We used development rate data of (1) eggs, (2) larvae feeding on a high-quality host, and (3) larvae feeding on a low-quality host growing at constant temperatures to estimate the parameter t. We followed three approaches, described above: the classic model, the I&T model, or an assigned
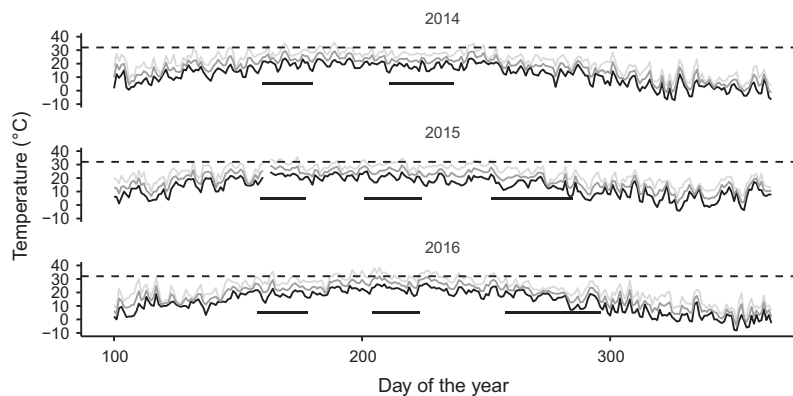
Figure 2 Curves indicate minimum (black), mean (grey), and maximum (light grey) daily temperatures according to weather station US1MDPG0009. Dashed lines indicate 32 °C, which was the upper critical temperature for all models. Solid lines indicate the periods when *Epargyreus clarus* caterpillars were in the field (from hatching to fourth instar). An early frost in 2014 killed third-generation larvae. The average minimum-maximum temperature range across years for each generation were 19.5–29.1 °C (generation 1), 20.2–29.6 °C (generation 2), and 14.3–23.4 °C (generation 3).

generic value of 10 °C (Figure 1). These combinations of data sources and modeling approaches resulted in a total of seven t values (Table 1).

We used each t value as the base temperature to estimate k, the number of degree days required for larvae to develop from hatching to fourth instar (k_{4th}). To calculate k, we used two sets of data, obtained from laboratory- or field-reared larvae. For each data set, we estimated three k values, for (1) larvae feeding on high-quality foliage (kudzu), (2) larvae feeding on low-quality foliage (wisteria), and (3) all larvae, disregarding foliage quality. As a result, each base temperature had six associated values of k resulting from the combination of two data sources (field- or laboratory-reared larvae), and three development time values, depending on host plant (high quality, low quality, or both; Table 1). For phenology forecasts without regard to host quality, a single k value was used to predict development time of all larvae; in contrast, when acknowledging host quality, two values of k were used, one for each host. We predicted development time of larvae in the field using two sets of k values, one derived from laboratory data (larvae growing at constant temperatures) and the other from field data (larvae growing under natural temperature regimes), which are commonly calculated when using generic t values. We compared field- vs. laboratory-derived k values with a t-test.

To compare the predictive ability of the models resulting from all of these combinations of t and k, we predicted the development time of seven generations of larvae growing in a common garden in the field. To generate development time predictions, we computed the days required for the accumulation of k degree days from the time each generation of caterpillars was deployed in the field to the time they reached the fourth instar, using weather data from station US1MDPG0009 (39.0443N, 76.8866W) located ca. 9 km from the common garden. To estimate degree-day accumulation, we applied a single sine function estimating daily temperature fluctuations using minimum and maximum daily temperature.

To assess model accuracy, we regressed observed mean larval development times on those predicted by each model and compared r^2 values across models. We discarded the first generation of 2014, as it was used to calculate field k. Models differed in the temperature interval within which they could be implemented (limited by the lower and upper critical temperatures), so we predicted only generations experiencing temperatures within the valid range. For most models this meant discarding the third generation, which developed in late summer and

Table 1 Components of degree-day models and the combinations of modeling approaches and data sets we followed to calculate them. We obtained seven values of t by applying each modeling approach – classic (ordinary least squares linear regression), generic (assigning a generic value of 10 °C), and I&T (major axis linear regression) – to three data sets: development rate of eggs (t_a, t_e), and larvae feeding on either a high- (t_b, t_f) or a low-quality (t_c, t_g) host. Each t was subsequently used to calculate four sets of k values, one for each combination of laboratory- vs. field-reared larvae feeding on a high- and low-quality host. The calculation of k is standard across modeling approaches

			Data source	
Degree-day model components	Modeling approach	$t_{model\ name}$	Life stage	Host quality
1. Valid interval Interval of temperatures within which temperature and development rate has a linear relationship, delimited by the upper and lower critical temperatures.	Classic	t_a	Eggs	
2. Base temperature (t) Temperature above which degree days accumulate		t_b	Larvae	High
		t_c		Low
	Generic	t_d	No data required, t = 10 °C	
	I&T	t_e	Eggs	
		t_f	Larvae	High
		t_g		Low

		Environment	
Degree-day model components	Host quality	Laboratory	Field
3. Degree-day sum (k) Number of degree days required to complete a life stage. We calculated six values of k_{4th} (degree days accumulated from hatching to fourth instar) for each t (a–g) depending on the combination of host and environment.	High	k_1	k_2
	Low	k_3	k_4
	Combined	k_5	k_6

early autumn, when average and minimum daily temperatures often dropped below 20 and 15 °C, respectively (Figure 2). Combining the three modeling approaches with development time data of larvae feeding on two host plants resulted in 26 sets of predictions encompassing the first two generations, and an additional 10 covering all three generations, for a total of 36 regressions of observed on predicted development time. The sample size of each of these regressions was 10 when evaluating only two generations per year (two observed development times, per each of five generations) and 14 when evaluating all three generations per year (two observed development times per each of seven generations).

To predict voltinism in the Maryland area, we calculated the degree-day accumulation required to complete development to reproductive maturity (k_{full}) in the laboratory. To calculate k_{full}, we summed the degree-day accumulation required for egg hatching in the laboratory (from laying to caterpillar hatching) and the degree-day accumulation from caterpillar hatching to adult emergence in the laboratory, as they came from different trials. Using these parameters, we calculated mean degree-day accumulation in the field from 1 May to 20 October over 3 years (2014–2016) and estimated the number of generations that could be completed within this period.

Results

Thermal biology of silver-spotted skippers and dependence on host plant quality

Eggs of *E. clarus* hatched when maintained at temperatures between 14 and 32 °C; however, larvae were able to complete development only at temperatures between 20 and 32 °C. First instars survived to molting under a broader range of temperatures (14–38 °C) than all other life stages (Figure 3). Only three temperature regimes permitted survival to adulthood (20, 26, and 32 °C) and although survival among these temperatures did not differ for caterpillars feeding within a given host (kudzu: $\chi^2 = 2.74$, P = 0.25; wisteria: $\chi^2 = 2.25$, P = 0.32, both d.f. = 2), larvae feeding on kudzu (high-quality foliage) were 24% more likely to survive than those feeding on wisteria, regardless of temperature treatment ($\chi^2 = 16.86$, d.f. = 1, P<0.0001; Table 2).

Egg development time varied from 5 days at 32 °C to 22.4 days at 14 °C (a more than four-fold difference); when larvae fed on kudzu, the duration of the full life cycle (egg to adult) ranged from 5 weeks at 32 °C to 11 weeks at 20 °C (Table 2). Feeding on wisteria delayed development by ca. 2 weeks in both temperature treatments. Larval development accelerated as temperature increased up to 32 °C, after which development slowed down
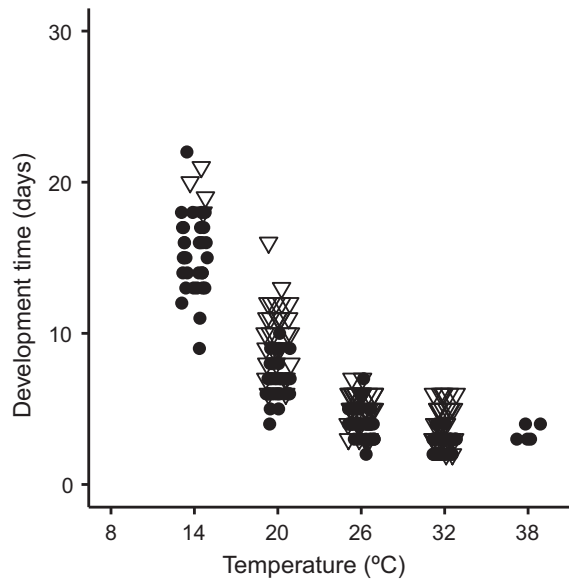
Figure 3 Development time (days) of first instars (hatch to first moult) of *Epargyreus clarus* on kudzu (circles) and wisteria (triangles). Points have been jittered for clarity. There are no points at 8 °C because all caterpillars died; at 38 °C only five caterpillars feeding on kudzu survived to second instar and then died.

(Figure 3). The incidence of supernumerary instars was significantly higher on caterpillars feeding on wisteria than on kudzu ($\chi^2 = 94.53$, d.f. = 1, P<0.0001; Table 2). Over 60% of the caterpillars feeding on wisteria exhibited six instars regardless of temperature ($\chi^2 = 3.61$, d.f. = 2, P = 0.16; Table 2), whereas only a small fraction (5%) of individuals feeding on kudzu exhibited six instars, all of which were kept at 32 °C (Table 2).

Thermal thresholds t and k

The set of linear regression approaches and data combinations that we used to calculate base temperatures yielded values of t ranging from 8.19 to 13.88 °C (Figure 4). Overall, t values estimated using the I&T model were larger than those based on the classic model (Table 3). However, prediction accuracy of models developed by these two approaches was very similar (Figure 5, Table 4). Estimation of the valid interval varied among modeling approaches and data sets (Table 4). Model a had the broadest valid interval and was constructed using egg data following the classic approach (Table 4). In contrast, models based on larval data had a more restricted range, between 20 and 32 °C, regardless of host quality and modeling approach (Table 4). Because development time was longer for larvae feeding on wisteria both in the laboratory (Table 2) and in the field (Figure 5), estimates of the

Table 2 Mean (± SE) development time of eggs, fourth instars, and egg to adult (days), survival from caterpillar hatching to adult emergence (%), and incidence of supernumerary instars of *Epargyreus clarus* under constant temperatures and in the field (average mean temperature across years). For field caterpillars the mean of each generation across years is reported. Numbers in parentheses indicate sample sizes (n)

| Host plant | Temperature | Development time (days) | | | Survival (%) | Incidence of supernumerary instars (%) |
		Egg	Hatch to fourth instar	Egg to adult		
Kudzu	8	–	–	–	0	–
	14	22.4 ± 0.56 (10)		–	0*	–
	20	9.1 ± 0.27 (15)	25.78 ± 0.43 (32)	76.06	80	0
	26	5.8 ± 0.10 (16)	15.29 ± 0.41 (36)	46.94	92.5	0
	32	4.9 ± 0.06 (18)	11.51 ± 0.18 (35)	36.93	87.5	5
	38	–	–	–	0	–
	Field I (24.3)		17.07 ± 0.47 (91)	–	–	–
	Field II (24.9)		21.14 ± 0.53 (96)	–	–	–
	Field III (18.8)		28.17 ± 1.24 (24)	–	–	–
Wisteria	8		–	–	0	–
	14		–	–	0	–
	20		53.52 ± 0.99 (23)	88.9	54	64
	26		25.88 ± 0.77 (32)	60.4	70	66.7
	32		20.79 ± 0.68 (29)	49.74	64	77.8
	38		–	–	0	–
	Field I (24.3)		22.45 ± 1.04 (58)	–	–	–
	Field II (24.9)		24.63 ± 0.91 (72)	–	–	–
	Field III (18.8)		30.95 ± 1.41 (21)	–	–	–

degree-day accumulation required for completing a life cycle (k) were also about 60% greater for individuals feeding on low- vs. high-quality foliage (Table 3). In addition, field and laboratory k values differed (t = −4.66, d.f. = 20, P<0.001; Table 3), although not in a uniform way. For example, field estimations of k corresponding to caterpillars feeding on the high-quality host were, in general, smaller than laboratory values, whereas the opposite was true for caterpillars feeding on the low-quality host (Table 2).

Field phenology and model predictions

Development time of larvae in the field varied among years and generations (year*generation: χ^2 = 50.032, d.f. = 3, P<0.0001; Figure 5). For the first generation of caterpillars in 2014, which we used to calculate field k_{4th} [development time from deployment in the field (0- to 1-day-old neonates) to the last date they were recorded as fourth instar], was (mean ± SE) 25.5 ± 0.76 days when feeding on wisteria, and 15.87 ± 0.44 when feeding on kudzu. Ambient temperatures experienced by the first two generations of caterpillars in all years were higher (mean >20 °C) than those experienced by the third generation (mean 14.3 °C), which developed at the end of the growing season, when minimum temperatures occasionally dropped below

10 °C (Figure 2). Because development rate and temperature have a linear relationship within a limited range, and degree-day models assume a linear relationship between these variables, we applied each model to predict only the phenology of larvae growing at ambient temperatures that fell within the corresponding temperature range. Most models (four out of seven) could therefore be applied to predict only the first two generations of skippers. In addition, phenological predictions for the third generation were less accurate than for the first two generations, as models underestimated larval development time on the low-quality host and overestimated it on the high-quality host (Figure 5). These over- and under-estimations were particularly large when implementing laboratory-calculated values of k (Table 4).

Considering predictions of the first two generations only, all models that incorporated variation due to host quality were consistently more accurate than those that did not (Table 4, Figure 5). Moreover, in most cases the relationship between observed and predicted development time was statistically significant only when acknowledging host quality (Table 4, Figure S2). Predictions developed through both I&T and classic modeling approaches to estimate t yielded similar r^2 values; however, implementing field-calculated instead of laboratory-calculated k resulted
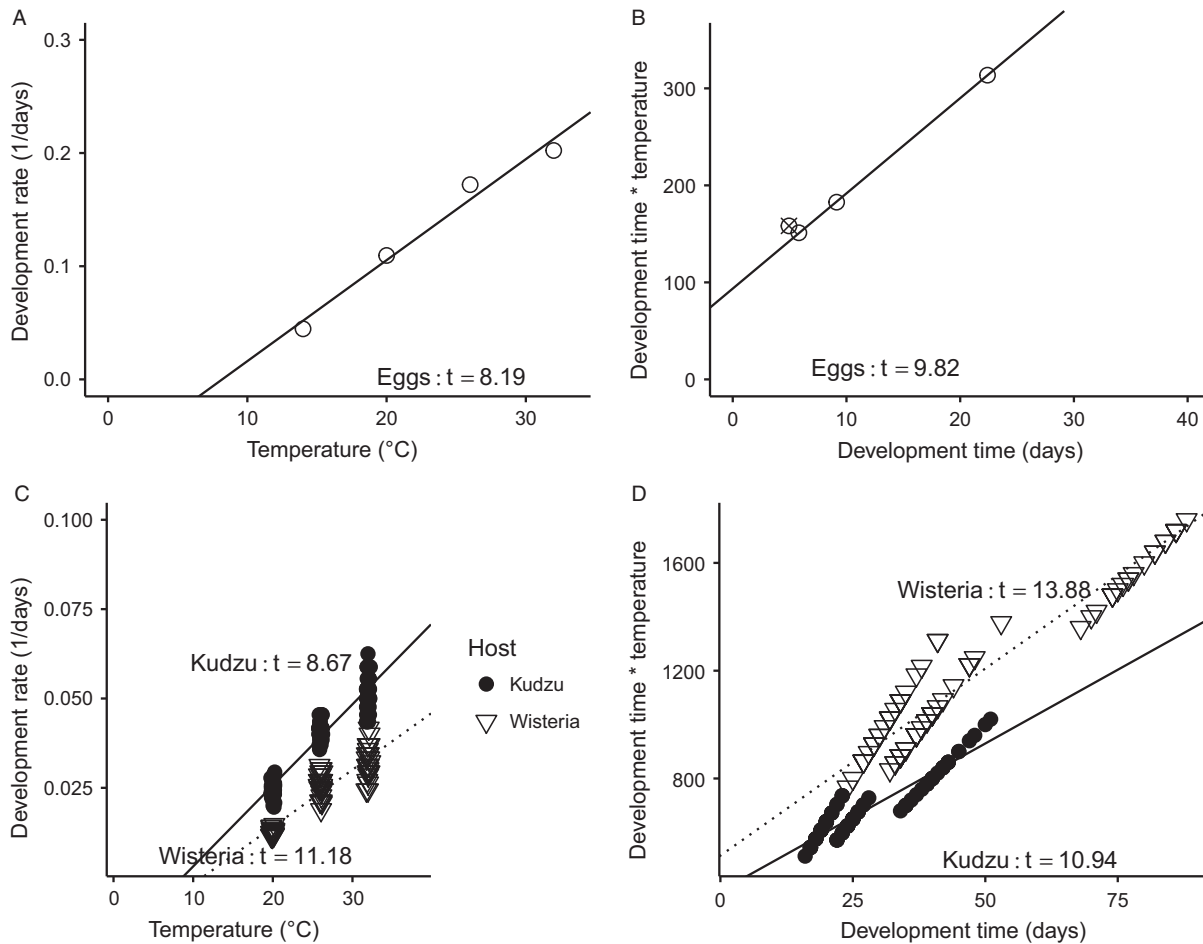
Figure 4 Temperature-dependent development of *Epargyreus clarus* and estimation of the lower development threshold t. The top panels indicate the estimation of t using egg data, following the (A) classic (R^2 = 0.977, $F_{1,2}$ = 83.3, P = 0.01; n = 4), and (B) I&T (r^2 = 0.999, P = 0.004; n = 3) approaches. The crossed data point in (B) was discarded. The bottom panels include the estimation of t using larval data following the (C) classic (kudzu: R^2 = 0.902, $F_{1,103}$ = 953.7, P<0.0001, n = 105; wisteria: R^2 = 0.811, $F_{1,73}$ = 313.4, P<0.0001, n = 75), and (D) I&T (kudzu: r^2 = 0.934, P<0.0001, n = 105; wisteria: r^2 = 0.964, P<0.0001, n = 75) approaches.

in slightly more accurate predictions of larval development (larger values of r^2; Table 4). Two models, c and g, provided the most accurate predictions for the first two generations. Model c resulted from applying the classic approach to larvae reared on the low-quality host and explained 53% of variation in caterpillar development time (Table 4). Model g resulted from applying the I&T model to data from caterpillars feeding on the low-quality host and explained 56% of variation in caterpillar development time (Table 4). However, these models could be used for predicting only the first two generations of field caterpillars, as they had restricted temperature ranges. Only three models (a, d, and e) could be applied to predict development time of all three generations (Table 4). When predicting all three generations, values of r^2 were greater when host quality was not included (Table 4), and

model a, the classic model applied to egg data, yielded the most accurate predictions. All models predicted that three generations of caterpillars could develop in the Maryland area when feeding on kudzu and only two when feeding on wisteria (Table 3).

Discussion

Our results demonstrate the importance of considering host plant quality when predicting phenological patterns of herbivorous insects. Indeed, accounting for variation in host quality had a larger effect on the accuracy of phenological predictions than did the choice of base temperature for calculating degree days (t). Incorporation of host quality into degree-day models significantly improved phenological predictions for larvae growing in early and mid-

Table 3 Lower temperature threshold (t) calculated by applying the classic model to three data sources, and degree-day accumulation (k) required to develop from hatching to fourth instar (k_{4th}) in the field, in the laboratory, and from hatching to life cycle completion in the laboratory (k_{full}). Three k_{4th} values were calculated for each threshold (t), depending on host quality: high, low, or both (pooling all individuals). Laboratory k values represent the average among temperature treatments. Voltinism was calculated for Maryland, USA, using the average degree-day accumulation within the period of 1 May to 20 October for the years 2014–2016

| Model		t (°C)	Host quality	Field k_{4th}	Lab k_{4th}	Lab k_{full}	Voltinism
a	Eggs	8.19	High	273.51	283.61	871.19	3
			Low	439.35	529.28	1206.45	2
			Both	335.45	406.44	–	–
b	Larvae reared on high-quality host	8.67	High	265.35	275.20	845.60	3
			Low	426.39	513.25	1170.42	2
			Both	325.37	394.22	–	–
c	Larvae reared on low-quality host	11.18	High	222.683	231.21	711.79	3
			Low	358.62	429.43	981.97	2
			Both	272.66	330.32	–	–
d	Generic	10	High	242.74	251.89	774.7	3
			Low	390.48	468.83	1070.56	2
			Both	297.44	360.36	–	–
e	Eggs	9.82	High	245.80	254.92	766.89	3
			Low	395.34	481.71	1074.49	2
			Both	301.22	368.31	–	–
f	Larvae reared on high-quality host	10.94	High	226.76	235.41	724.59	3
			Low	365.1	437.44	999.98	2
			Both	277.70	336.43	–	–
g	Larvae reared on low-quality host	13.88	High	176.78	183.88	567.86	3
			Low	285.62	339.27	779.25	2
			Both	215.96	261.58	–	–

summer, but not at the end of the season. The poor performance of models when predicting the third generation was likely due to larval exposure to stressful low temperatures and diapause-inducing cues. This generation faces unique developmental trade-offs because it overwinters – these trade-offs significantly affect development time (Dmitriew, 2011) and can override the effect of host plant.

Both temperature and host quality affected egg and larval survival and development time. Eggs are typically more resilient to low temperatures than larvae (Bowler & Terblanche, 2008). *Epargyreus clarus* eggs developed into larvae under a broader temperature range (14–32 °C) than that under which larvae developed successfully into pupae (20–32 °C); thus, models using egg data to calculate t allowed for predictions at lower ambient temperatures. Whereas 32 °C minimized development time in our study, it also triggered the appearance of an extra instar, indicating stress. Extra instars were not triggered at 26 °C; therefore, we anticipate the optimum temperature for *E. clarus* development to be between 26 and 32 °C, which encompasses previously reported values for other temperate butterflies (31–33.6 °C; Bryant et al., 2000). Feeding on a high-quality host increased survival by as much as 86%

under non-lethal temperatures and prolonged the time individuals were able to endure lethal temperatures at both extremes. These findings suggest that an herbivore's choice of host plant could affect its ability to withstand spells of unfavorable weather. Poor host quality and low temperature slowed larval development in the field and the laboratory.

Differences in host quality were large enough to result in differences in number of generations prior to diapause. All of our models predicted the occurrence of three complete generations when feeding on kudzu but only two when feeding on wisteria in the Maryland area, a difference with significant evolutionary implications. (In the common garden, we grew three generations on both hosts because larvae were introduced to the garden at the start of each generation from a laboratory colony, so the completion of one generation was not necessary to start the next). Further studies will test whether *E. clarus* exhibits host-specific patterns of voltinism.

The comparative approach used in our study allowed us to evaluate different methodologies to estimate degree-day model parameters t and k. We evaluated three approaches (classic, I&T, generic value) to calculating t, and found that
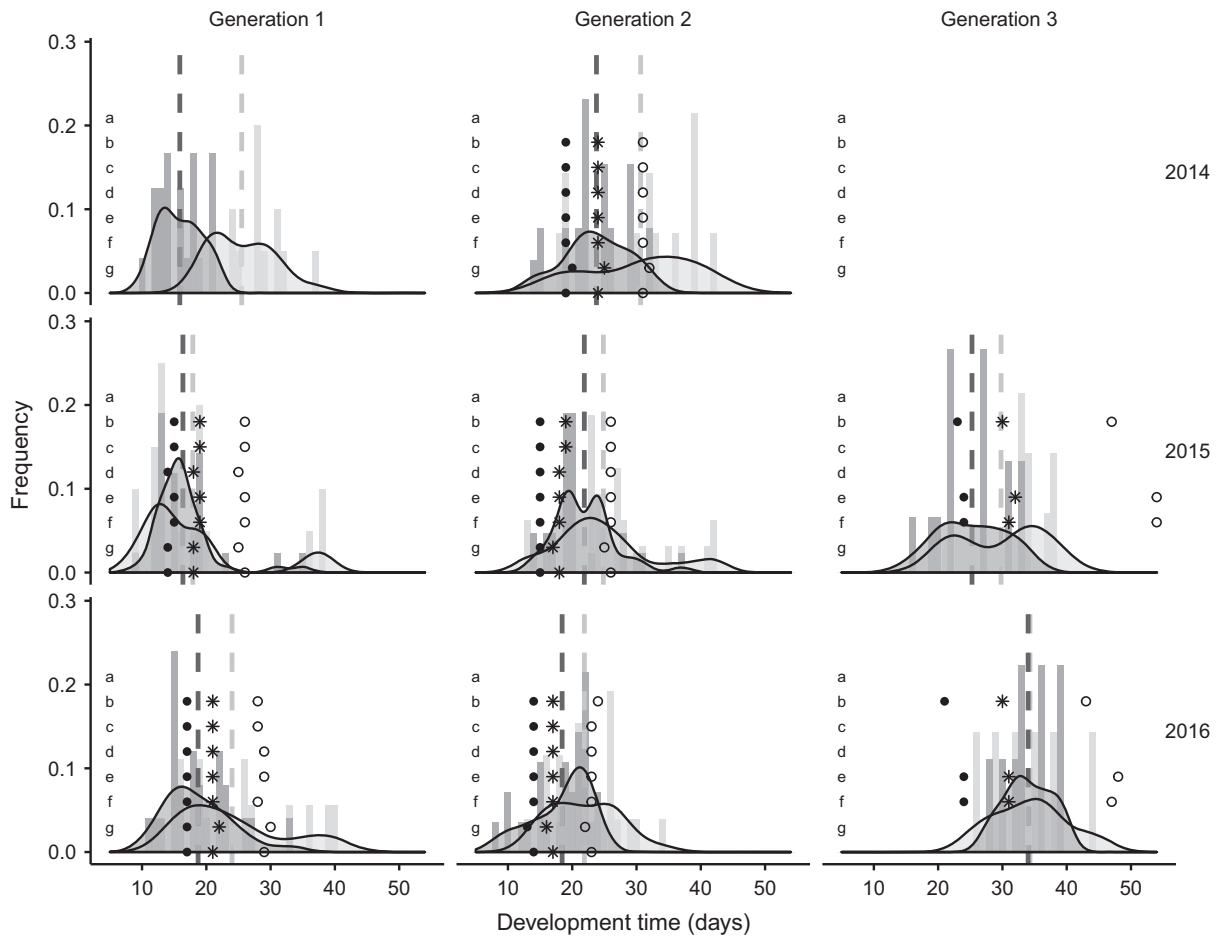
Figure 5 Observed and predicted field phenology of three *Epargyreus clarus* generations (columns) over 3 years (rows). Observed phenology is expressed using histograms, where bars indicate the frequency of development times of fourth instars feeding on high-quality (kudzu, dark grey) and low-quality (wisteria, light grey) hosts. Density plots have been overlaid to highlight peak values and distribution spread. Mean development time is indicated with a dashed line for each host (black for kudzu, grey for wisteria). Letters on the left correspond to the various models (see Table 4) and are horizontally aligned with their corresponding predictions, black dots for kudzu, white circles for wisteria, and stars for both hosts combined (note there are no predictions for generation 1 of 2014, as it was used to predict the other seven generations). All of these predictions were calculated using field-calculated k values. For an analogous figure based on laboratory-calculated k values see Figure S1.

no model is superior with regard to accuracy of predictions. All three approaches to calculate t yielded accurate and similar results when predicting the first two generations; however, they were considerably less accurate for the third generation, for which they overestimated development time on low-quality wisteria by a minimum of 4 weeks (linear regression) to as much as 7 weeks (I&T and generic estimation methods). Whereas variation in t had little effect on prediction accuracy, methodological differences in estimating k had a large impact on model fit. Models based on field-calculated k consistently outperformed those using laboratory-calculated k, which is not surprising because the former incorporate variation in

other physiologically relevant environmental conditions such as relative humidity and temperature oscillation.

The poor predictive performance of the models in the third generation was likely a result of larval exposure to stressful low temperatures and diapause-inducing cues. Larvae developing at the end of the season are typically exposed to low temperatures, a decreasing photoperiod, and reduced host quality, all of which act as cues for diapause induction and can alter development time (Hunter & McNeil, 1997; Takagi & Miyashita, 2008). Host quality declines as the season advances, as mature leaves have both lower water and N content than young leaves (Hunter & Lechowicz, 1992). Photoperiodic conditions typical of the

Table 4 Comparison of models implemented to predict development time of larvae in the field. Parameter t was obtained by applying the classic linear regression model to each data source across the valid temperature interval (°C). Values of k correspond to either the degree days accumulated by the first generation of larvae of 2014 (from first to fourth instar) in the field or by laboratory-reared larvae, at the corresponding base temperature t. Values of r^2 correspond to the regression between predicted and observed development time of larvae in the field when predictions were calculated either acknowledging (by host) or disregarding (both) host quality. Corresponding F and P values are included. Because only a subset of models could be implemented to predict all three generations, we report first r^2 values of all models predicting generations I and II, and then the two models that could also predict the third generation. There are no laboratory k values for the generic model, as it is developed without laboratory data

Generations	Data source	Valid interval (°C)	t	k source		r^2	Significance		
							F	d.f.	P
I and II	a Eggs	14–32	8.19	Field	By host	0.500	7.99	1,8	<0.05
					Both	0.403	5.41	1,8	<0.05
				Laboratory	By host	0.436	6.17	1,8	<0.05
					Both	0.317	3.72	1,8	0.09
	b Larvae reared on high-quality host	20–32	8.67	Field	By host	0.509	8.30	1,8	<0.05
					Both	0.403	5.41	1,8	<0.05
				Laboratory	By host	0.436	6.17	1,8	<0.05
					Both	0.317	3.72	1,8	0.09
	c Larvae reared on low-quality host	20–32	11.18	Field	By host	0.532	9.09	1,8	<0.05
					Both	0.385	4.99	1,8	0.056
				Laboratory	By host	0.477	7.29	1,8	<0.05
					Both	0.356	4.49	1,8	0.067
	d None, generic t value applied	–	10	Field	By host	0.497	7.90	1,8	<0.05
					Both	0.326	3.88	1,8	0.085
	e Eggs	14–26	9.82	Field	By host	0.509	8.30	1,8	0.020
					Both	0.326	3.88	1,8	0.084
				Laboratory	By host	0.444	6.40	1,8	0.035
					Both	0.317	3.72	1,8	0.09
	f Larvae reared on high-quality host	20–32	10.94	Field	By host	0.504	8.11	1,8	0.022
					Both	0.385	4.99	1,8	0.056
				Laboratory	By host	0.469	7.06	1,8	0.029
					Both	0.360	4.49	1,8	0.067
	g Larvae reared on low-quality host	20–32	13.88	Field	By host	0.559	10.12	1,8	0.013
					Both	0.297	3.38	1,8	0.103
				Laboratory	By host	0.559	10.12	1,8	0.013
					Both	0.395	10.12	1,8	0.051
I, II, and III	a Eggs	14–32	8.19	Field	By host	0.471	10.69	1,12	<0.01
					Both	0.668	24.18	1,12	<0.001
				Laboratory	By host	0.461	10.26	1,12	<0.01
					Both	0.576	16.28	1,12	<0.01
	d None, generic t value applied	–	10	Field	By host	0.503	12.13	1,12	<0.01
					Both	0.618	19.39	1,12	<0.001
	e Eggs	14–26	9.82	Field	By host	0.503	12.14	1,12	0.005
					Both	0.641	21.44	1,12	0.001
				Laboratory	By host	0.292	4.54	1,12	0.056
					Both	0.543	14.27	1,12	0.003

end of the season can significantly reduce development times and size at maturity (reviewed in Dmitriew, 2011). When predicting the third generation, disregarding host quality actually increased prediction accuracy, because using the average of both hosts to predict development time mitigated the overestimation that occurred for larvae feeding on wisteria and the underestimation that occurred for larvae feeding on kudzu. Phenological predictions for overwintering larvae could be improved by accounting for diurnal oscillation in both temperature and photoperiod. In addition, understanding how thermal stress affects the compensatory responses of caterpillars feeding on

suboptimal host plants may result in better host-customized thermal units (e.g., host-specific values of k).

Accurate phenological predictions of the last generation are critical for conservation purposes, as this generation largely determines the size of the overwintering population, which, if reduced, may result in population declines. This drawback is likely less of a concern for pest control, as crops are usually restricted to the period with favorable environmental conditions, and region-specific values for t and k are often estimated for each species, thus accounting for local variation in weather.

Overall, we demonstrate that accounting for variation in host quality when calculating k is more important than the choice of development threshold t. Degree-day models developed from standard values of t and field-calculated k provided acceptable phenological predictions, especially when incorporating host plant quality information. Thus, this method was the most efficient option we tested, when balancing accuracy and effort. Generalist and oligophagous species account for up to a quarter of all herbivores, and they are more common at temperate latitudes (Lill et al., 2002; Forister et al., 2015), where the effects of climate change are also more pronounced (IPCC, 2014). Thus, it is important to understand how the abundance, distribution, phenology, and quality of host plants change in response to climate, as opposed to focusing solely on the direct effects of temperature on insect development. In conclusion, our work highlights both the importance of acknowledging host plant distribution and use when predicting lepidopteran phenology, and the need to develop strategies to manage the uncertainty increments observed under stressful circumstances, which are likely to be more prevalent under predicted global climate change scenarios.

Acknowledgements

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Supporting Information