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LETTER

Compensating for climate change–induced cue-environment mismatches: evidence for contemporary evolution of a photoperiodic reaction norm in *Colias* butterflies

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Abstract

Anthropogenic climate change alters seasonal conditions without altering photoperiod and can thus create a cue-environment mismatch for organisms that use photoperiod as a cue for seasonal plasticity. We investigated whether evolution of the photoperiodic reaction norm has compensated for this mismatch in *Colias eurytheme*. This butterfly's wing melanization has a thermoregulatory function and changes seasonally. In 1971, Hoffmann quantified how larval photoperiod determines adult wing melanization. We recreated his experiment 47 years later using a contemporary population. Comparing our results to his, we found decreased melanization at short photoperiods but no change in melanization at long photoperiods, which is consistent with the greater increase in spring than summer temperatures recorded for this region. Our study shows that evolution can help correct cue-environment mismatches but not in the same way under all conditions. Studies of contemporary evolution may miss important changes if they focus on only a limited range of conditions.

Keywords

Climate change, *Colias eurytheme*, contemporary evolution, evolutionary trap, melanization, phenotypic plasticity, photoperiod, reaction norm, seasonal mismatch.

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INTRODUCTION

Seasonal variation in weather, resources and other environmental factors is ubiquitous, and many organisms have evolved seasonal plasticity in a wide range of traits to adapt to this variation (Bradshaw 1965; Shapiro 1976; Tauber *et al.* 1986). Seasonal plasticity, like all adaptive plasticity, relies on the ability to use cues from the current environment to reliably predict the future environment (Levins 1963; Bradshaw 1965; Moran 1992). Photoperiod is among the most commonly used seasonal cues, particularly in temperate and polar regions where it is a critical cue for many aspects of phenology (Dawson *et al.* 2001; Bradshaw & Holzapfel 2007; Walton *et al.* 2011; Nylin 2013; Tang *et al.* 2016). Photoperiod can also provide a cue for seasonal plasticity of other traits, such as colour pattern, which is strongly influenced by photoperiod in species ranging from snowshoe hare to many butterflies (Shapiro 1976; Mills *et al.* 2013). Photoperiod is a common cue because it remains constant from year to year without being affected by short-term weather fluctuations. This consistency often allows photoperiod to predict future seasonality more reliably than current temperature or other factors (Bradshaw & Holzapfel 2007). Many organisms rely on the predictable, stable relationship between photoperiod and seasonal conditions, so what happens when this relationship changes?

Anthropogenic climate change has altered many aspects of the environment, not only raising temperatures, but also increasing their variability, altering both meteorological and biological seasonality and ultimately changing key aspects of ecology such as species ranges and community composition (IPCC 2014; Walther *et al.* 2002; Parmesan & Yohe 2003). For example, in many regions mean winter and spring temperatures have increased more than summer temperatures (IPCC 2013), leading to greater phenological changes for species active earlier in the year (Walther *et al.* 2002; Menzel *et al.* 2006; Calinger *et al.* 2013; Ovaskainen *et al.* 2013). Climate change has also led to phenotypic changes for many organisms (MacLean *et al.* 2018), the most common of which is declining body size (Gardner *et al.* 2011). However, photoperiod itself does not change with climate change. This invariance alters the relationship between photoperiod and seasonality, creating a mismatch where the photoperiod which previously predicted one thermal or other environment now indicates different conditions. In turn, this less accurate cue will produce inappropriate plastic responses in animals that use photoperiod as a predictor of seasonality. Numerous examples of these climate-change induced mismatches exist for the timing of phenological events, particularly breeding in birds (Visser *et al.* 2004; Visser & Both 2005; Renner & Zohner 2018), but mismatches can also occur in other seasonally plastic traits. For example, snowshoe hare, *Lepus americanus*, continue to change from summer to winter coloration at a consistent date despite later snowfall and earlier snowmelt; by mid-century this is predicted to impair their camouflage for weeks (Mills *et al.* 2013).

Like any other trait, photoperiodic reaction norms can evolve given sufficient genetic variation (e.g. Groeters & Dingle 1987; Śniegula*et al.* 2014), and thus selection could correct for these new mismatches by altering the response to photoperiod. Unfortunately, documenting adaptive evolution in response to climate change-induced cue-environment mismatches has proven difficult, and most studies have not

distinguished between genetic and plastic responses (Gienapp *et al*. 2008). Although not feasible for many systems, the ideal way to detect these changes is to recreate past laboratory experiments which directly assess a species response to photoperiod. Bradshaw & Holzapfel (2001) used this approach to detect changes in the critical photoperiod that induces diapause in the pitcher-plant mosquito, *Wyeomyia smithii* between 1972 and 1996. A similar approach has also been used to detect evolution of hatching date in the eggs of winter moths, *Operophtera brumata*, between 2000 and 2010, but using temperature as a cue instead of photoperiod (Van Asch *et al.,* 2013). Here we adopt this approach to investigate evolutionary changes in the seasonal plasticity of a new trait, colour pattern, in *Colias eurytheme* butterflies over nearly five decades.

Colias eurytheme, the orange sulphur, is a polyvoltine butterfly with a photoperiod-dependent seasonal polyphenism. Many species of *Colias* and other Pierid butterflies display seasonal plasticity in wing colour pattern (Shapiro 1976). This polyphenism has been shown to aid thermoregulation in multiple species, including *C. eurytheme*; specifically, cool-season *C. eurytheme* have greater melanization on the basal ventral hindwings which raises body temperatures by increasing absorption of solar radiation (Watt 1969; Kingsolver & Wiernasz 1991). Since the early 1970s, multiple studies of *C. eurytheme* have demonstrated that the melanization of the butterfly's ventral hindwings is primarily determined by larval photoperiod during the third and fourth instars and not influenced by temperature (Hoffmann 1973; Jacobs & Watt 1994). *C. eurytheme* uses larval photoperiod as a cue because of how it reliably predicts the future thermal environment experienced by the emerging adult 2–3 weeks later (Hoffmann 1978). Photoperiod also influences the body size of *C. eurytheme,* with smaller individuals at short photoperiods, although the shape of the reaction norm differs from colorations (Hoffmann 1973). Thus, *C. eurytheme* is a prime example of a species which relies on the predictive relationship between photoperiod and seasonal temperature change, making it vulnerable to the cue-environment mismatch created by climate change. Previous work has demonstrated evolution of the thermal performance curve for feeding in *C. eurytheme* larvae over the last 40 years in response to climate change (Higgins *et al*. 2013), indicating that *C. eurytheme* has the potential to evolve on this time scale.

In this study, we tested for evolutionary change in the photoperiodic reaction norm of *C. eurytheme* by recreating Hoffmann (1973), the original study which identified photoperiod as the cue for seasonal plasticity in this species. In 1971, Hoffmann (1973) raised the offspring of a central California *C. eurytheme* population under a range of photoperiods (and temperatures) and measured the melanization of the ventral hindwing of the resulting adults. We conducted the same experiment in 2018 on *C. eurytheme* from a nearby location, testing for changes in the reaction norms of both hindwing melanization and forewing length (as a proxy for body size). We hypothesized multiple potential changes which could correct for the cue-environment mismatch. (1) The butterfly could become brighter overall, independent of photoperiod, indicated by increased reflectance at all photoperiods. (2) The butterfly could separately change its warm or cool season phenotype, indicated by altered reflectance only under long or short photoperiods respectively. (3) The butterfly could shift the photoperiodic threshold at which it switches between forms, indicated by altered reflectance only at intermediate photoperiods.

METHODS

Study organisms

Colias eurytheme Boisduval is a widespread polyvoltine species found throughout most of North America. In much of its range *C. eurytheme* hybridizes with *Colia sphilodice* and *Colia seriphyle* (Hovanitz 1949; Jahner*et al*. 2011), but neither of these other species occur in the Central Valley of California, where our study focused, so no hybridization occurs there (Dwyer *et al*. 2015). In the Central Valley, the flight season of *C. eurytheme* is typically from March to December with short, overlapping generations after a relatively synchronized first generation (Hoffmann 1973). In the laboratory at 25 °C, a single generation from egg to adult takes 23 days, with about 12 days between the photoperiod sensitive period and adulthood (Hoffmann 1973). Outside the flight season, *C. eurytheme* overwinters as larvae or pupae, but without entering diapause (Hoffmann 1974; Schweitzer 2006; Jahner*et al*. 2011).

For our study, we caught adult female *C. eurytheme* from a patch of roadside flowers adjacent to an alfalfa field near Isleton, California, United States (38.154°N, 121.677°W, 0 m) on 26 August 2018. At this location, day length varies from 9.50 to 14.87 h per day (10.50–15.87 h including civil twilight). This location was about 1°N and about 140 km from Los Banos, California (37.06°N, 120.85°W, 36 m), the source of the butterflies used by Hoffmann (1973), and the habitat (low elevation alfalfa fields in an agricultural landscape) was very similar. *Colias eurytheme* can disperse long distances (Schweitzer 2006), and previous research found little evidence for genetic population structure across a similar distance in California (Dwyer *et al*. 2015). We consider genetic differentiation in our focal trait between these locations unlikely, but If there was any differentiation it should be the opposite direction of our predicted response to climate change (darker individuals further north), making our analysis conservative. Hoffmann (*pers. comm*) used *Vicia villosa* as a host plant for his experiments, which we also used. Because Hoffmann (1973) only provided data for male *Colias eurytheme,* we likewise focused on males in this study. Hoffmann reported no qualitative differences between the plasticity of males and females, and we found a largely similar response to photoperiod in males and females of contemporary populations (see Supplemental Materials).

Rearing conditions and experimental design

We sought to replicate Hoffmann (1973) as closely as possible, using the same methods and conditions as reported by Hoffmann whenever feasible (Table S1). Females were kept chilled during transit and in the laboratory in Chapel Hill,

North Carolina until the start of egg laying. Wild-caught females had already mated. To obtain eggs, females were placed individually in clear plastic cups with fresh sprigs of *V. Villosa* for 3 h and heated with an incandescent light. Only offspring of females which laid at least 50 eggs were used in the experiment to ensure sufficient caterpillars from each female to split among all treatments. Under the conditions described above, all but one female laid > 50 eggs, and seven families were ultimately used in and tracked through the experiment.

Eggs were kept on the *V. Villosa and* they were laid on in a growth chamber at 25 °C and a 12:12 light: dark cycle. Upon hatching, each family was divided evenly among five photoperiod treatments ranging from 10:14 to 14:10 light: dark in 1-hour increments, all at 25 °C. Hoffmann (1973) tested the full range of photoperiods from 0 to 24 h of light, but we focused on the photoperiods that best covered the plastic response to natural photoperiods. Hoffmann used an unknown number of 4-watt fluorescent lights, whereas our growth chambers used four 32-watt fluorescent lights. Low light intensities are typically sufficient to induce photoperiodic responses (Lees 1956), so differences in light intensity are unlikely to affect our results. Hoffmann (1973) started his photoperiod treatments as eggs; however, he also identified the third and fourth larval instars as the sensitive period for photoperiodic cues and showed that the photoperiod experienced as an egg had little or no effect on adult phenotype. An additional group of caterpillars from the same families was set up in the 14:10 treatment for later use in pupal temperature manipulation (see below). Caterpillars were fed daily fresh 3- to 5-week-old, greenhouse-grown *V. villosa ad libitum*. However, at the end of larval development (September 14-16, 2018), some caterpillars ran out of food between feedings due to lack of greenhouse access during Hurricane Florence; any associated food stress occurred across all treatments and was unassociated with photoperiod. Initial caterpillar densities were at most 25 per dish, and they were rapidly decreased as caterpillars developed to a final maximum density of three per dish during late fifth instar. *C. eurytheme* has an endemic polyhedrosis virus transmitted through the egg which can lead to high mortality under laboratory conditions (Steinhaus 1948; Tanada*et al.* 1964), particularly during late larval instars and pupation. This disease was present and caused substantial mortality during Hoffmann's experiment (pers. comm.), and in our experiment resulted in greatly reduced final numbers that were not evenly distributed across treatments (Final sample sizes: 10L: n = 31, 11L: n = 16, 12L: n = 28, 13L: n = 27, 14L: n = 17).

Pupae remained in their growth chamber and were moved to plastic cups for emergence once they had hardened (2 days post-pupation). The exception was pupae in the temperature manipulation treatment, which were moved to a separate growth chamber at 20 °C and 14:10 light:dark. Hoffmann (1973) performed his temperature manipulation on the 16:8 light:dark treatment, which was not in our experiment; however, he found no difference between 14:10 and 16:8, indicating that these photoperiods are comparable; the minimal differences we found reinforce this (see results). Hoffmann did not specify the exact timing with which he moved the pupa when he manipulated temperature, only stating 'Newly pupated insects at 25 °C on 16 h of light per day were allowed to complete development and emergence at 15, 20, 25, and 30 °C'. Thus, to ensure any temperature effect did not depend on when the pupae were moved, they were checked twice daily and moved either as soon as we observed signs of pupation (a fifth instar caterpillar which had attached itself to the dish with silk or a newly molted pupa) or 1 day later to provide time to harden. These two cases did not differ significantly in hindwing melanization (t-test, $t_{33} = 0.89621$, $P = 0.377$), so we pooled them for later analysis.

Once butterflies emerged, they were frozen in glassine envelopes to euthanize them for later phenotypic measurements.

Photography of wings

To measure the colour pattern and size of the wings, we dissected all wings from each butterfly's body and photographed them. All four wings, a tag with a specimen id number and a colour standard (Image Science Associates ColorGauge Pico Target, Matte version) were placed on a grey craft-foam background (Creatology, gray foam sheet). Two photographs were taken, one on the dorsal side of the wings and one on the ventral side. Photographs were taken with a Canon Rebel EOS T6s digital SLR camera using a Canon EF 100 mm macro lens, mounted on a copystand with the end of the lens 34 cm above the specimen and operated remotely with EOS Utility (Canon, v3). Illumination was primarily from a Canon Macro Twin Lite MT-24EX paired flash, with white tissue paper to diffuse the shadows. Although some ambient light was present, the colour standard allowed for correction of that variation (see below). Photographs were taken with manual settings: ISO 100, aperture f/11, and exposure time 0.5 s; focus was automatic.

Image processing and measurement

As a first step, images were converted from the original RAW files to TIFF format with a neutral colour balance (i.e. removing the automatic colour balancing performed by the camera) using RawTherapee (v5.2). Subsequent image processing was conducted using custom programs written for that purpose using primarily the OpenCV package (v3.2.0; Bradski 2000) in Python (v3.6.2). We standardized each colour channel in the images with a regression of the mode of each grey scale patch on the colour standard to expected values provided by the manufacturer. The value of r exceeded 0.998 for all colour channels in all images, indicating the quality and reliability of this correction. The colour standard also provided a scale for the image. We identified the outline of each wing using the colour difference between the wings and the grey background. Visual inspection confirmed correct identification of the wings in all images, except for some wrinkled or otherwise damaged wings (see below). As a measurement of hindwing colour, we calculated the mean value of the linearized red and green colour channels over the entire ventral hindwing. For forewing length, we used the distance between the two furthest apart points on the forewing. For analysis, we averaged the measurements from both ventral hindwings, although we excluded wings which could not be properly measured because they

were wrinkled or otherwise damaged (this led to the full exclusion from the colour measurement of one butterfly at 11L:13D and one at 14L:10D). See supplementary materials for a more detailed description of our computational methods.

Comparison to historical measurements

We obtained Hoffmann's original measurements by using the Figure_Calibration plugin (Hessman 2009) for ImageJ (v1.52a; Schneider *et al*. 2012) to estimate means and standard errors from Figures 3 and 4 in Hoffmann (1973). Hoffmann measured reflectance at 650 nm. To compare our measurements to his, we measured the reflectance of a random subset of 10 specimens from each treatment (see supplemental materials). The photographic and reflectance measurements were highly correlated ($r = 0.938$). We used their regression to create a conversion function and estimate reflectance measurements à la Hoffmann of all individuals from their photographs. For graphical comparison, we converted his standard errors to 95% confidence intervals by multiplying them by 1.96. Hoffmann (1973) did not provide sample sizes for his photoperiod experiments. For statistical comparison, we assumed a sample size of 10 for each treatment based on his reported sample sizes for temperature manipulation. When comparing Hoffmann's data to ours, we first used the known mean and standard error along with the assumed sample size to construct a surrogate data set with the same properties, following Larson (1992), which could then be compared to our data using a two-way ANOVA for the effects of photoperiod treatment (as a factor) and year (1971 vs. 2018), with and without the interaction term. We also tested other assumed sample sizes from 2 to 30 (Table S2); the overall effect of time period remained significant for all tested sample sizes, and the interaction between time period and photoperiod was also significant or nearly significant for most of this range. Information on the response to temperature came from Table 1 in Hoffmann (1973), which provided means, 95% CI and sample sizes. We statistically compared the results of our temperature manipulation to Hoffmann's results using the same methods as for photoperiod, except using the known sample sizes. All statistical analysis was conducted in R (v3.4.4).

To more precisely analyse how reflectance and wing length in the contemporary population responded to photoperiod, we used a mixed model with photoperiod as a fixed effect and family as a random effect. We did not know the family of one individual, who was excluded from the mixed model analysis (exclusion or inclusion of this individual made no notable difference to our results). Comparison of within and between family variation indicates that we have sufficient families to estimate population mean values (Supplemental Materials, Pinheiro & Bates 2006). Models were fit using lmer in the lme4 package (Bates *et al*. 2015), and *P*-values generated by a likelihood ratio test comparing the full model to one without photoperiod.

Historical changes in environmental temperatures

To quantify how the environmental temperature experienced by *C. eurytheme* in this area has changed over the past 50 years, we compared weather station temperature data from the decade prior to each study (Hoffmann: 1962–1971, current study: 2009–2018). These data came from the nearest National Oceanographic and Atmospheric Administration weather station to our present-day collection site that had data covering the full date range (Station USC00040232: lat = 37.983°N, long = 121.753°W, elevation = 18.3 m; distance from collection site = 20 km). We extracted data of daily maximum and minimum temperatures for 1 month from each season (March, July, October). We used two-way ANOVA to test for an interaction between month and historical time period (decade) on both minimum and maximum daily temperature.

RESULTS

We measured reflectance from a total of 117 individuals from seven families reared across five photoperiod treatments. Hindwing reflectance (at 650 nm) varied significantly with developmental photoperiod in contemporary populations, increasing under longer photoperiods (χ^2_4=122.87, $P < 2.2 \times 10^{-16}$; Figure 1). Contemporary butterflies had on average 0.027 higher reflectance than in Hoffmann's study ($F_{1,161} = 15.45$, $P = 0.00013$); however, this difference varied with photoperiod ($F_{4,157} = 2.87$, $P = 0.025$; Figure 1). At short photoperiods, mean reflectance has increased (0.038, 0.065 and 0.031 at 10, 11 and 12 h light, respectively), to the extent that 95% confidence intervals did not overlap at 10 h light, and only overlapped minimally at 11 and 12 h light (Figure 1). By contrast, confidence intervals overlapped substantially at longer photoperiods, and the mean reflectance was nearly unchanged (0.007 and −0.003 at 13 and 14 h light respectively). The range of mean hindwing reflectance from 10 h light to 14 h light has decreased by 25% since 1971 from 0.16 to 0.12 because of this increased reflectance under short photoperiods.

We measured forewing length from 119 individuals. Like reflectance, forewing length varies significantly with developmental photoperiod in contemporary populations, increasing with longer photoperiods ($\chi^2_4 = 23.99$, $P = 8.0 \times 10^{-5}$; Figure 2). Unlike reflectance, neither overall wing length

Table 1 Comparison of the effect of pupal temperature on reflectance of *Colias eurytheme* hindwings at 650 nm between 1970/1971 and 2018

	1970/1971			2018			
Pupal temperature (°C)	N	Mean reflectance	95% CI	N	Mean Reflectance	95% CI	Difference between means
20	12	0.440	0.022	35	0.436	0.009	−0.004
25	8	0.455	0.018	16	0.455	0.013	0.000
Difference between means		0.015			0.019		

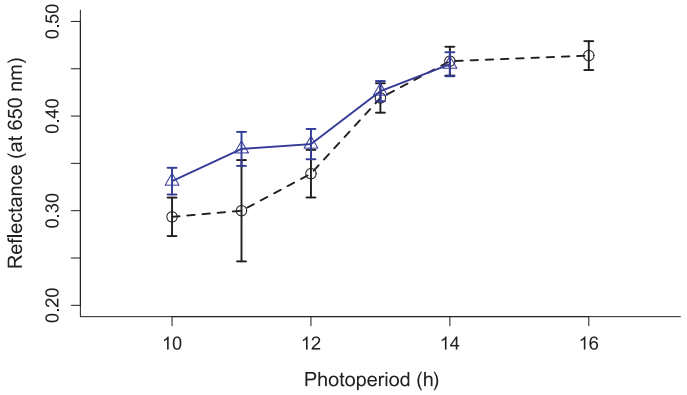

Figure 1 Reflectance (proportion of light reflected) at 650 nm of male *Colias eurytheme* ventral hindwings when raised from hatchingat different photoperiods in either 1970/1971 (black circles, dashed line)[28] or 2018 (blue triangles, solid line). In both cases all individuals were raised at 25°C. Measurements from 2018 are estimated from digital photographs of wings (see Methods). Bars represent 95% CI.

($F_{1,163}$ = 0.286, P = 0.59) nor the response of wing length to photoperiod ($F_{4,159}$ = 0.789, P = 0.53) has changed significantly between 1971 and 2018.

Using 51 pupae from seven families kept at different temperatures, we found a small but significant effect of temperature on reflectance (β = 0.018, $F_{1,69}$ = 5.98, P = 0.017). Mean reflectance was 0.018 higher at the higher pupal temperature (Table 1), 15% of the overall effect of photoperiod in contemporary populations. This effect did not, however, change significantly between 1971 and 2018 (β = 0.0037, $F_{1,67}$ = 0.052, P = 0.82), nor did reflectance overall change significantly across years (β = −0.0025, $F_{1,68}$ = 0.108, P = 0.74). Given the lack of interaction between year and temperature, increased power due to our larger sample size is the most likely explanation for why we found a significant effect of temperature on melanization when Hoffmann did not. Comparing field temperatures from the decade before each experiment, the change in mean daily maximum temperature varied significantly among months ($F_{2,1597}$ = 7.41, P = 6.2 × 10^{-4}, Figure 3a). Specifically, March warmed by 1.96 °C, while July and October only warmed by 0.31 °C and 0.45 °C respectively. Minimum temperatures increased between time periods even more than maximum temperatures, and again the difference varied significantly between months ($F_{2,1591}$ = 13.38, P = 1.7 × 10^{-6}, Figure 3b). For minimum temperatures, July again warmed the least (2.60°C), but October warmed the most (4.33 °C) and March in between (3.46 °C).

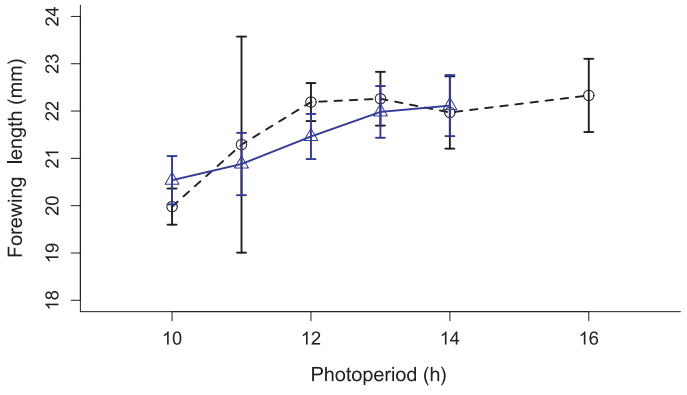

Figure 2 Forwing length (mm) of male *Colias eurytheme* when raised from hatching at different photoperiods in either 1970/1971 (black circles, dashed line)[28] or 2018 (blue triangles, solid line). In both cases all individuals were raised at 25°C. Bars represent 95% CI.

DISCUSSION

We found evidence for evolution of *C. eurytheme*'s photoperiodic reaction norm over the last 47 years, but only at short photoperiods. Offspring of butterflies collected in 2018 and raised as larvae at photoperiods between 10 and 12 hours of light per day had brighter ventral hindwings, a key trait for thermoregulation, than those raised under the same photoperiods and similar laboratory conditions by Hoffmann in 1971. This change corresponds to greater climate change in spring than summer at our collection site. Hoffmann (1973, 1978) was able to use his laboratory results to accurately predict the phenotypes collected in the field across the year. As noted, the photoperiodic reaction norm evolved in the direction we

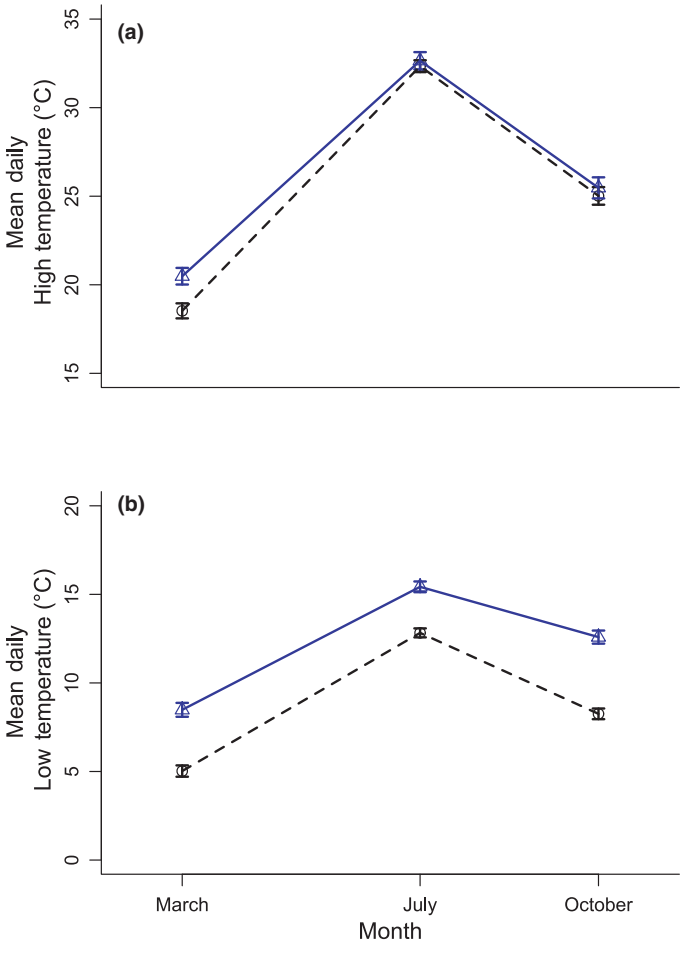

Figure 3 Mean daily (a) high temperature and (b) low temperature near collection site during three months (March, July, October) from 1962 to 1971 (black circles, dashed line) and 2009 to 2018 (blue triangles, solid line). Bars represent 95% CI.

would predict under anthropogenic climate change, but the phenotype did not change at all photoperiods. Adult *C. eurytheme* have become brighter under short photoperiods corresponding to spring and fall, but not the long, summer photoperiods. This largely corresponds to the changes in temperature which have occurred in this region over the same time period: spring daily high temperatures have warmed considerably while summer temperatures have increased little if at all. These patterns also correspond to a well-established broader trend that cool seasons are warming more than hot seasons (Bradshaw & Holzapfel 2008; IPCC 2013). Daily low temperatures also increased more than daily highs, indicating a greater change in selection by low temperatures, strongest during the spring, than selection by high temperatures, strongest during the summer. This difference could also contribute to the greater evolutionary change at short photoperiods. Notably, we observed little change in fall maximum temperature, which could represent a constraint on the evolution of simple photoperiodic responses. Any spring photoperiod also occurs in the fall, so if these periods change differently, evolution cannot optimize the response to both unless additional information is available. In particular, some insects use directional change in photoperiod (increasing or decreasing) to distinguish spring and fall and produce appropriate plastic phenotypes (Tauber *et al.* 1986; Nylin 2013). Both our and Hoffmann's larvae were reared at constant photoperiods, and future work could test whether *C. eurytheme* uses change in photoperiod to differentiate between seasons despite their short sensitive period. Limitation of how bright *C. eurytheme* can become could be a final explanation for the uneven change in response to photoperiod. Variation in the brightness of ventral hindwings in *Colias* occurs primarily through changes in the number of melanic scales present (Hoffmann 1973), creating a limit to how bright a *Colias* butterfly can become. Nevertheless, this limit has probably not been reached because the reflectance of a pure yellow portion of the wing remains higher than what we observed under long photoperiods (Kingsolver 1983). Because we were not able to raise multiple generations in the laboratory, we cannot entirely rule out maternal effects or epigenetic inheritance as the source of some of the differences between our results and Hoffmann's. Nevertheless, Hoffmann was able to predict field phenotypes quite accurately using only the photoperiodic response curve from his laboratory-reared butterflies (Hoffmann 1973; Hoffman 1978), indicating that any trans-generational effects on melanization are likely small. Additionally, to create our results, these trans-generational effects would have to change the developmental plasticity of colour, rather than colour itself. Although parental effects on the coloration of hatchlings are well demonstrated for desert locusts (Islam *et al.* 1994; Elliot *et al.* 2003; Tanaka & Maeno 2006), we are not aware of any studies showing trans-generational effects on adult coloration in insects, much less changing how adult colour responds to developmental cues.

We reconstructed Hoffmann's studies (1973) as closely as possible but were limited by details missing from the original manuscript. In particular, the sample sizes of the original photoperiod-manipulation experiment are unknown. Nevertheless, the change in response to photoperiod we found should be robust given that the overall effect of time was significant across the full range of sample sizes we tested, and the overlap in 95% confidence intervals varied from none at 10 h light to almost entirely at 13 and 14 h light. Another difference was that we were not able to acquire individuals from the exact same location as Hoffmann (1973). Nevertheless, our site had a similar habitat (low-elevation alfalfa fields), and given that our site was slightly north of Hoffmann's, we would have expected *a priori* for it to have darker individuals, the opposite of the pattern we observed. Our measurement method also differed from Hoffmann (1973); however, the strong correlation between measurement methods allowed us to correct for this difference in our analysis. Other aspects of our experiment may not have been completely identical to Hoffmann (1973) — for example containers used for rearing larvae were not the same, and we were unable to maintain completely *ad libitum* feeding for the final few days of the experiment. However, in the unlikely case that these factors did affect melanization, they should have affected all photoperiods, a pattern we did not see. Instead, we observe a change in the photoperiodic reaction norm that corresponds closely to the observed climate change in ways that most methodological differences would be unlikely to produce.

At the same time, we did not detect changes in two other reaction norms that Hoffmann (1973) also studied: the response of wing size to photoperiod, and the response of hindwing melanization to temperature. Our results for wing length are less clear than those for wing colour. Although we found no significant interaction between photoperiod and year in determining body size, our statistical analyses were limited by the unknown sample size of Hoffmann's study. Regardless, we found that *C. eurytheme* continues to use photoperiod as a cue for seasonal body size variation, even though another study failed to detect this effect in 1994 (Jacobs & Watt 1994).

Overall, *C. eurytheme* is a widespread species, found throughout most of North America. Seasonal plasticity and the cues underlying it may vary greatly across this range because of natural geographic variation in temperature and photoperiod, as well as climate change. Future work can investigate this variation over multiple populations, which may also help us understand the relative contributions of standing genetic variation, genetic drift and gene flow to the evolutionary changes we found. In other parts of its range, *C. eurytheme* co-occurs and hybridizes with other *Colias* species (Jahner*et al.* 2011), at least one of which is known to respond quite differently to photoperiod and temperature (Hoffmann 1978). Hybridization could thus be another source of genetic variation in seasonal plasticity for other populations of *C. eurytheme*, leading to quite different responses to climate change.

Our research highlights both the difficulty and value of replicating older, historical studies. Population distributions, environmental conditions and many other factors change such that the same conditions as the original study will never truly exist (Shavit & Ellison 2017; Ives 2018). Here we show how, in the face of this variation, best-possible replication of past studies can document how results may have changed due to these different circumstances. We emphasize the importance of clear documentation of methods, data and analysis for replicability of research (Fidler *et al.* 2017).

We have found evidence for contemporary evolution of a photoperiodic reaction norm corresponding to the cue-environment mismatch caused by climate change. This evolutionary change only altered the phenotype at certain photoperiods, corresponding to the times of year which experienced the greatest change. Given the broad interest in phenotypic and evolutionary responses to climate change (Gienapp *et al*. 2008; Visser 2008; Huey *et al*. 2012; Schilthuizen& Kellerman 2013; Maclean *et al*. 2018; Kelly 2019), our results indicate that changes found at certain times of year may not occur in others, an important limitation to studies that only look at traits under a limited range of seasonal or other conditions. This result shows the potential for independent evolution of different parts of the reaction norm instead of evidence for constraint by genetic correlation across environments, addressing a classic question about the evolution of plasticity (Via & Lande 1985; de Jong 1995; Via *et al*. 1995). We also show how evolution of the shape of a reaction norm can help correct for an anthropogenic cue-environment mismatch. Such 'evolutionary traps' can be created by a wide range of anthropogenic changes in addition to climate change and pose serious threats to the stability and survival of populations and species (Schlaepfer*et al*. 2002; Robertson *et al*. 2013; Van Dyck et al. 2014). Studies like ours which recreate historical experiments measuring reaction norms could be used to assess whether evolution can compensate for the cue-environment mismatches created by a wide range of additional anthropogenic changes, so long as the historical data are available. Studies of contemporary trait evolution need to pay attention to the cues which influence trait plasticity because they may be changing quite differently from selection on the trait itself.

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AUTHOR'S CONTRIBUTIONS

Both authors designed the study. MEN conducted the experiments and analysed the data. MEN drafted the manuscript, and both authors contributed to editing and approved the final manuscript.

Data Availability Statement

Data, python code and R scripts used in this study are available from the Dryad Digital Repository at: https://doi.org/10.5061/dryad.m0cfxpp0q

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SUPPORTING INFORMATION

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

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