Received: 1 April 2022 | Accepted: 20 September 2022

DOI: 10.1111/icad.12608

SHORT COMMUNICATION

Insect Conservation and Diversity

Royal Entomological Society

Effects of temperature on monarch caterpillar pigment variation in nature

Michelle Tseng[1,2] | Carolina Bevanda[1,2] | Sahibveer Singh Bhatti[1,2] | Emily N. Black[1,2] | Elizabeth Chang[1,2] | Jonathan Chiang[1,2] | Harleen Dhaliwal[1,2] | Alexandra Dimitriou[1,2] | Sunny Y. Gong[1,2] | Eashan Halbe[1,2] | Noam Harris[1,2] | Lydia Huntsman[1,2] | Jennifer Ann Lipka[1,2] | Juniper Malloff[1,2] | Erin McHugh[1,2] | Mira Mikkelsen[1,2] | Armin Noroozbahari[1,2] | Amelija Olson[1,2] | Daniel Pirouz[1,2] | Kishoore Ramanathan[1,2] | Maryann Rogers[1,2] | Suman Singh[1,2] | Jenna Rachel Skurnac[1,2] | Samantha Straus[1] | Yolanda Sun[1,2] | Yu Jia Sun[1,2] | Grace Wang[1,2] | Justin Kwong Ching Wong[1,2]

[1]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

[2]Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence
Michelle Tseng, Departments of Zoology and Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
Email: tsengm@mail.ubc.ca

Editor: Karsten Schönrogge and
Associate Editor: Francesca Barbero

Abstract

1. Insect colour patterns serve a wide range of ecological functions and the biotic and abiotic factors mediating colour variation in nature have been well characterised.

2. Nonetheless, the majority of studies in this field have focused on adult insects (particularly butterflies). Almost nothing is known about the factors that mediate intra-specific colour variation in juveniles in nature, even though they are often as conspicuously coloured as their adult counterparts.

3. Here we show that temperature predicts a small but significant amount of monarch (*Danaus plexippus*) caterpillar pigment variation in nature. Over a 650,000-km^2 region in Canada and the USA, caterpillars found in warmer locations or lower latitudes had thinner black stripes than those found in colder locations or higher latitudes. Caterpillars have also become less black over the last five years, a result consistent with observed short-term increases in summer temperature in this region.

4. Our study demonstrates that the relationship between temperature and monarch caterpillar pigmentation seen in laboratory settings is also apparent in nature, although with considerable variation. Our study also highlights the utility of online biodiversity repositories such as iNaturalist for characterising pattern and colour variation in nature.

KEYWORDS
caterpillar, colour, iNaturalist, monarch butterfly, pigment, temperature

INTRODUCTION

Insect colour patterns serve a wide range of functions including acting as warning signals to predators, aiding in camouflage, attracting mates and helping with thermal regulation (Davis et al., 2007; Mello et al., 2022; Willadsen, 2022; Yumnam et al., 2021). Because of the clear link between colouration and fitness, several of the biotic and abiotic factors that mediate intraspecific variation in colour pattern have been characterised. In lepidopterans, where the bulk of this research has been conducted (Davis, 2014), rearing density altered pupae colour in the butterfly *Mycalesis mineus* (Mayekar & Kodandaramaiah, 2021), but did not affect wing pigmentation in the wood tiger moth *Parasemia plantaginis* (Nokelainen et al., 2013) or in the monarch butterfly *Danaus plexippus* (Atterholt & Solensky, 2010). With respect to abiotic factors, among other examples, geometrid moths were darker at higher latitudes (Heidrich et al., 2018), Himalayan butterfly *Pieris canidia* was darker at higher elevations (Gautam & Kunte, 2020) and drier habitats produced darker larvae of the moth *Chiasmia clathrata* (Välimäki et al., 2015).

Temperature in particular appears to have clear effects on intraspecific variation in lepidopteran colouration (Hill et al., 2021). Higher growing temperatures resulted in lighter colouration in African armyworm (*Spodoptera exampta*) larvae, Asian comma (*Polygonia caureum*) pupae and speckled wood (*Parage aegeria*) adults (Aguilon et al., 2015; Taylor-Cox et al., 2020; Yamanaka et al., 2012). Similarly, monarch larvae reared in the laboratory at warmer temperatures displayed thinner black stripes compared to those reared at colder temperatures (Davis et al., 2005; Solensky & Larkin, 2003). Increased melanisation in colder temperatures is presumed to be adaptive because darker pigmentation allows for greater absorption of thermal energy and therefore faster development (Hill et al., 2021; Solensky & Larkin, 2003; True, 2003). Despite the importance of insect colouration for fitness, tests of the relationship between temperature and larval pigmentation have primarily been conducted in controlled laboratory settings (Davis et al., 2005; Sandre et al., 2007; Solensky & Larkin, 2003; Välimäki et al., 2015). Investigating the relative importance of temperature in nature can help us to better understand geographic variation in larval patterning and better predict the potential effects of ongoing climate warming on insect populations.

Monarchs are an ideal species in which to examine caterpillar pigment variation in nature because their conspicuous black, white and yellow stripes make them easy to identify (Figure 1a), and tens of thousands of monarch caterpillar photos are freely available on biodiversity repositories such as iNaturalist (iNaturalist.org). In this study, we quantified monarch caterpillar pigmentation (percent black) from photos available on iNaturalist. We hypothesise that caterpillar pigment is affected by temperature in nature. Based on results from laboratory studies, we predicted: (a) that caterpillars observed in warmer locations would have thinner black stripes, which (b) should result in a latitudinal gradient in caterpillar pigmentation with thicker black stripes at higher latitudes; and (c) if temperatures in our study region have increased over the study period (2017–2021), caterpillars observed more recently should have thinner black stripes.

METHODS

Photo acquisition

Monarch caterpillar photos were sourced from iNaturalist (inaturalist.org), a free online repository of biodiversity images collected by iNaturalist users around the world. According to the *life stage* analysis on iNaturalist, the majority of monarch caterpillars in Canada and the United States are observed between July and September each year. Additionally, the *history* analysis on the website revealed that the bulk of the monarch observations on the website were uploaded after 2016. Accordingly, we filtered our search to include only caterpillars observed in July and August, and between 2017 and 2021. We also limited our search to *Research Grade* observations made in Ontario and the following US states: New York, New Jersey, Pennsylvania, Ohio and Maryland. *Research Grade* observations are those whose identifications have been confirmed by at least two iNaturalist users. Misidentified organisms can still obtain the *Research Grade* label, but given the unique patterning of monarch caterpillars, we believe that none of the caterpillars used in this study were misidentified. We chose this geographic region because it contained the highest density of monarch butterfly observations in iNaturalist. Consequently, we thought that this region would provide the highest number of photographs of caterpillars that were amenable to quantifying pattern variation. Coincidentally, adult monarchs from this region also make up ~20%–25% of the overwintering adult population (Flockhart et al., 2017).

Each year from 2017 to 2021, the same search query was used to call observations. For example, for 2019, the query was: https://www.inaturalist.org/observations?d1=2019-07-01&d2=2019-08-31&order=asc&order_by=observed_on&page=40&place_id=39,42,31,48,51,6883&quality_grade=research&taxon_id=48662. In total, this search resulted in approximately 28,000 caterpillar photos from 2017–2021, and covered approximately 650,000 km^2 or 12% (estimated using the measuring tool on Google Maps) of the breeding range.

Photo selection

We manually selected photos with the following conditions: the caterpillar was in focus, at least three abdominal segments appeared to be in a straight line and not twisted or curved (e.g. Figure 1a), the caterpillar was not shrivelled and there were sharp distinctions between each band. An example of a caterpillar with non-distinct stripes is included in Figure S1. We neglected to keep track of the number of photos that included caterpillars with non-distinct stripes. To estimate the approximate number of *fuzzy-striped* caterpillars on iNaturalist, we conducted an ad hoc search of 6309 larvae from this region and found the prevalence of *fuzzy-striped* larvae to be approximately 0.1%.

The total number of monarch caterpillar observations in iNaturalist has increased over time and ranged from approximately 1700 in 2017 to over 8000 in 2021. Of these available photos, we haphazardly chose between 102 and 142 caterpillar photos per year ($n = 109$, 142, 138, 109, 102 from 2017 to 2021, respectively), with approximately half of the photos taken in July, and half in August, per year. The sample size is not evenly distributed among years because some photos were out of focus upon closer inspection. We also only chose 5th instar caterpillars and used the length of the tentacles relative to the width of the caterpillar to distinguish between 4th and 5th instars (Oberhauser & Kuda, 1997) Specifically, in 5th instars, the front tentacles are approximately three times as long as the width of the

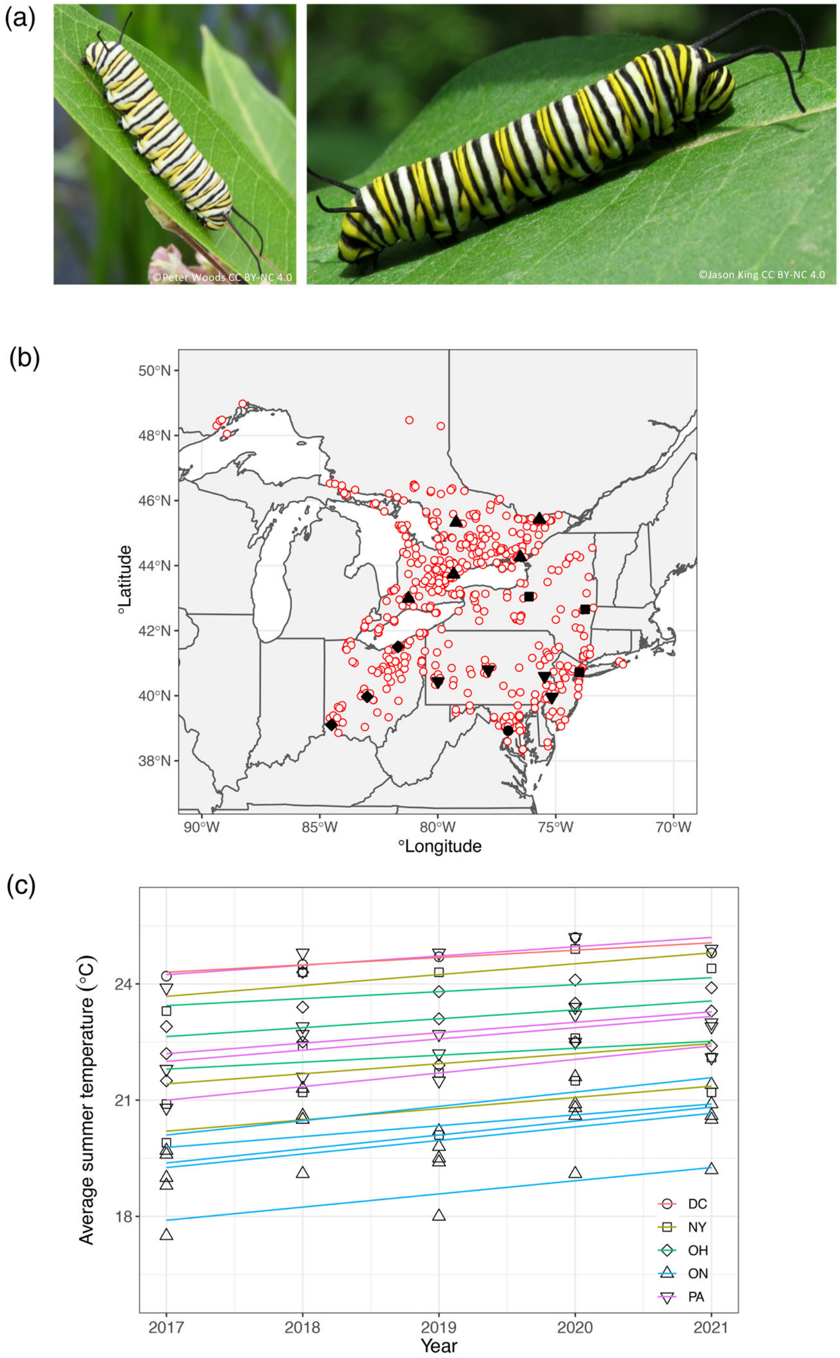

FIGURE 1 (a) Sample iNaturalist photos of monarch caterpillar larvae. L: observation 7006531 Peter Woods (CC BY-NC 4.0), R: observation 7441495 Jason King (CC-BY-NC 4.0). (b) Map of locations where caterpillars were observed (red circles) and of cities (black shapes) used to quantify regional changes in summer temperature from 2017 to 2021. Shapes are grouped by State or Province using the same key as in (c). Complete list of cities is available in Table S1. (c) Mean summer temperature from 2017 to 2021 for 16 cities located throughout the study region. Lines represent best fit lines from linear regressions of average summer temperature over time. Cities (Table S1) are grouped by State or Province. DC, District of Columbia; NY, New York; OH, Ohio; ON, Ontario; PA, Pennsylvania

head capsule but in 4th instars, the front tentacles are approximately twice as long as the width of the head capsule (Oberhauser & Kuda, 1997). This method is not foolproof and may have resulted in the inclusion of a few 4th instar larvae, which have slightly thinner black stripes compared to 5th instars (Oberhauser & Kuda, 1997). Nonetheless, we have no reason to believe that iNaturalist users from specific latitudes, locations, or years would preferentially upload photos of 4th instar caterpillars compared to iNaturalist users at other latitudes, temperatures or years and thus we do not believe that any of the relationships between caterpillar pigmentation and temperature or latitude observed here could be driven by the inadvertent inclusion of 4th instar larvae.

Quantifying caterpillar percent black

We used ImageJ (Rasband, 2016) to quantify caterpillar percent black. We first used the polygon selection tool to outline one caterpillar segment (Oberhauser & Kuda, 1997). Second, we used the measure function to measure the area in this segment (in pixels). Third, we used the polygon selection tool and measure function to measure the area of the black stripes within this segment. We divided the sum of the area of all of the black stripes by the area of the total segment to obtain the percentage of black per segment. We repeated these measurements for two additional segments and took the average percentage black of the three segments. We call this metric *percent black*. Although most of the photos were taken of the lateral view of the insect ($n = 449$), we also measured some dorsal-view photos ($n = 151$). The average percent black of lateral-view photos was significantly lower (mean ± standard deviation $= 38.1\% \pm 8.47$) than that of dorsal-view photos ($41.8\% \pm 8.1$; $F_{1,598} = 22.4$, $p < 0.0001$) and thus we included *view* as an explanatory factor in the statistical models. Because there are no scale bars on iNaturalist photos, we do not have data on absolute values of the areas of the black segments. Each author except SS and MT measured approximately 21 randomly assigned caterpillars.

Latitude, year and weather variables

We obtained latitude and longitude data for each observation from iNaturalist and we mapped all observations (Figure 1b). We also recorded the date the caterpillar was observed. We used the MS Windows 10 version of ClimateNA (Wang et al., 2016) to retrieve the average, maximum and minimum summer temperatures for individual observation locations during the year of collection. We used summer weather data because all of the caterpillars were observed in the temperate summer. Average, maximum and minimum summer temperatures were all highly correlated so we used average summer temperature as our metric of weather. We also used ClimateNA to obtain the average summer temperatures of 16 major cities throughout the study area (New York: Albany, New York City, Syracuse; Ohio: Cincinnati, Cleveland, Columbus; Ontario: Huntsville, Kingston, London, Ottawa, Toronto; Pennsylvania: Allentown, Philadelphia, Pittsburgh; State College; Washington DC, Figure 1b). We plotted the average summer temperature of each of these cities from 2017 to 2021 to examine short-term trends in summer temperature in this region.

Statistical analyses

We used linear regression to examine whether average summer temperature, latitude or year significantly explained variation in percent black of monarch caterpillars ($n = 600$). Because temperature is significantly correlated with latitude, and also with year, we ran one model with just temperature, and a second model with latitude, year and the interaction between latitude and year. Both models also included *photo view* (dorsal vs. lateral) as an additional explanatory variable. Because proportion data can sometimes violate assumptions of linear models, researchers advocate that Beta regression or Dirichlet regression should be used to analyse this type of data (Douma & Weedon, 2019). Here we used linear regression because plots of the raw data, residuals vs fitted data and normal q-q plots all confirmed that the data met assumptions of linear models (Figure S2). No data were transformed. We also used linear regression to examine short-term trends in average summer temperature for the 16 cities throughout the study region. All analyses were conducted in R version 4.0.4 (R Core Team, 2021).

RESULTS

Short-term changes in summer temperature in the study region

Average summer temperature in cities throughout the study region increased from 2017–2021 (year: $F_{1,48} = 45.6$, $p < 0.0001$; Figure 1c), and the 16 cities differed in their mean summer temperature (city: $F_{1,48} = 61.7$, $p < 0.0001$). The rate of increase in temperature did not differ among cities (year:city $F_{15,48} = 0.14$, $p = 0.99$; full model $r^2 = 0.92$). Averaged across cities, the rate of increase in temperature between 2017 and 2020 was 0.27°C per year, for a total increase of 1.35°C during the study period.

Effect of temperature on caterpillar percent black

Summer temperature had a significant effect on percent black ($F_{1,597} = 18.1$, $p < 0.0001$, slope $= -0.69$, model $r^2 = 0.06$). Caterpillars found in warmer locations displayed lower percent black compared to those found in cooler locations (Figure 2a). The effect of *photo view* was also significant ($F_{1,597} = 20.2$, $p < 0.0001$).

Effect of latitude and year on caterpillar percent black

There was a significant latitudinal gradient in caterpillar pigmentation ($F_{1,595} = 17.3$, $p < 0.0001$, slope $= 0.63$), with caterpillars observed in lower latitudes exhibiting lower percent black compared to caterpillars found in higher latitudes (Figure 2b). There was also a significant effect of year on percent black ($F_{1,595} = 14.7$, $p = 0.0001$, slope $= -0.97$; Figure 2c), with more recently-observed caterpillars displaying thinner black stripes compared to earlier observations. The interaction between latitude and year was not statistically significant ($F_{1,595} = 0.4$, $p = 0.53$), and the overall model r^2 was 0.07. The effect of *photo view* was also significant ($F_{1,595} = 17$, $p < 0.0001$). A few of the caterpillar observations were located above 47.5°N. The significant effects of latitude and year remain unchanged if these points are removed.

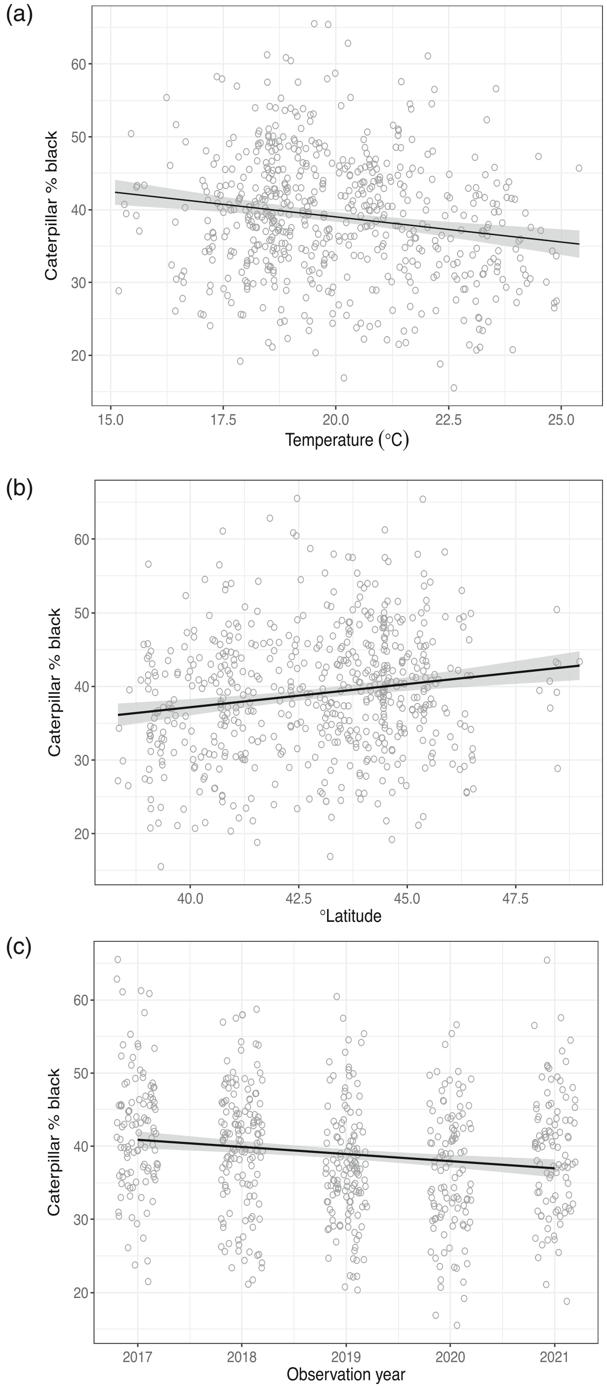

FIGURE 2 Relationship between caterpillar percent black and (a) mean summer temperature, (b) latitude and (c) year of observation. All relationships are statistically significant. The significant relationship between latitude and percent black remains if the points above 47°N are omitted. See Results for statistical analyses. Points in (c) are jittered to reduce overlap. Shaded areas represent 95% confidence intervals.

DISCUSSION

The goal of this study was to examine monarch caterpillar pattern variation in nature. We tested the general prediction that caterpillars should have less black pigmentation in warmer locations. We sourced monarch caterpillar photos from the online biodiversity repository iNaturalist and found that caterpillars observed in warmer locations, lower latitudes and more recently, displayed thinner black stripes compared to those observed in colder locations, higher latitudes and earlier in the 5-year study period.

This study is unique in that we document a small but significant role of temperature in mediating caterpillar patterning in nature. Although much is known about colour variation in adult butterflies or other insects (Caglar et al., 2014; Günter et al., 2020; Heidrich et al., 2018; Kozlov et al., 2021; Taylor-Cox et al., 2020), few if any studies have examined medium-to-large scale spatio-temporal pigment variation of caterpillars in nature (but see Black et al., 2021). With respect to monarchs in particular, previous studies have shown that caterpillar banding patterns are correlated with larval development and adult colouration, and that adult monarch colouration itself is correlated with flight distance (Davis et al., 2012; Hanley et al., 2013) and mating success (Davis et al., 2007). Thus, our results could have implications for monarch population dynamics. Experiments that directly link temperature-mediated shifts in monarch caterpillar pigmentation to population growth rates are needed to better understand the broader implications of variation in larval patterning.

The patterns observed here could also have arisen if more recent iNaturalist observations had been recorded from warmer locations or lower latitudes. We ran linear regressions to check these associations and found no evidence that caterpillar observations had shifted to warmer locations ($F_{1,496} = 0.02$, $p = 0.89$), or lower latitudes ($F_{1,598} = 0.44$, $p = 0.51$) between 2017 and 2021.

Additionally, although the effects of temperature and latitude seen here are statistically significant, they explain a very small fraction of the total variation in caterpillar percent black (6%–7%), suggesting that other important biotic and abiotic factors also affect the degree of caterpillar pigmentation in nature. For example, adult monarchs show sex-specific black banding patterns (Davis et al., 2005) and thus it is likely that male and female caterpillars too are dimorphic. Unfortunately, it is not possible to identify the sex of individual caterpillars via photographs. It is also unclear how much of the variation in pigmentation is due to genetics versus phenotypic plasticity, and given that monarchs belong to one large admixed population (Lyons et al., 2012; Talla et al., 2020), the degree of independence among the 600 measured individuals is also unknown. Interestingly, the explanatory power of temperature on caterpillar percent black is very similar to that of temperature on insect body size in nature (Kelemen & Rehan, 2021; Polidori et al., 2020; Tseng et al., 2018).

Our characterisation of monarch caterpillar pigment variation in nature was made possible by the thousands of photos uploaded to iNaturalist by scientists and the general public. This user-friendly and accessible biodiversity repository is quickly becoming an invaluable tool for the study of conservation (Light et al., 2021; Mesaglio et al., 2021), movement ecology (Supp et al., 2021), invasive species (Young et al., 2021), behavioural ecology (Clark et al., 2021), phenology (Jaskuła et al., 2021) and science outreach (Niemiller et al., 2021). Here we add to this list of applications by quantifying insect pigment variation in nature.

In summary, we have demonstrated using the iconic monarch butterfly that temperature has small but potentially important effects on caterpillar pigmentation in nature. Our study only encompassed 5 years of data ($n = 600$) and thus we refrain from speculating on how ongoing global warming may affect monarch fitness via changes in caterpillar pigmentation. Future studies that examine the adaptive significance of temperature-mediated pigmentation in nature, as well as the decadal-scale effects of temperature on caterpillar pattern variation will be important for predicting the effects of continued global warming on monarch butterfly population dynamics.

AUTHOR CONTRIBUTIONS

Michelle Tseng: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (equal); project administration (lead); writing – original draft (lead); writing – review and editing (lead). **Carolina Bevanda:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Sahibveer Bhatti:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Emily N. Black:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Elizabeth Chang:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Jonathan Chiang:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Harleen Dhaliwal:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Alexandra Dimitriou:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Sunny Y. Gong:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Eashan Halbe:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Noam Harris:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Lydia Huntsman:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Jennifer Ann Lipka:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Juniper Malloff:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Erin McHugh:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Mira Mikkelsen:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Armin Noroozbahari:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Amelija Olson:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Daniel Pirouz:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Kishoore Ramanathan:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Maryann Rogers:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Suman Singh:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Jenna Skurnac:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Samantha Straus:** Data curation (supporting); formal analysis (supporting); methodology (supporting). **Yolanda Sun:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Yu Jia Sun:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Grace Wang:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Justin Kwong Ching Wong:** Data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

UBC-Vancouver is situated on the traditional, unceded and ancestral lands of the Musqueam. The authors are grateful to the many Indigenous Nations on whose ancestral lands these monarch caterpillars were observed. They also thank the users of iNaturalist for their beautiful caterpillar photos and iNaturalist for maintaining this invaluable resource. They would also like to thank Andy Davis, two anonymous reviewers and the editorial team at ICAD for their many insightful comments on the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data are available in the UBC Dataverse Collection At Borealis: https://urldefense.com/v3/__https://borealisdata.ca/dataset.xhtml?persistentId=doi:10.5683*SP3*OYSFZP__;Ly8!!N11eV2iwtfs!r9UmFVJJBACIpMdFptlBjp7hxgSMdrn2VgIxkaTR-pQLCAHmnQiHKLhmDhPc2k6o8dBZs1NnLgCMDQ$

ORCID

Michelle Tseng https://orcid.org/0000-0002-4306-507X

REFERENCES

Aguilon, D.J.D., Medina, C.dR. & Velasco, L.R.I. (2015) Effects of larval rearing temperature and host plant condition on the development, survival, and coloration of African armyworm, *Spodoptera exempta* Walker (Lepidoptera: Noctuidae). *Journal of Environmental Science and Management*, 18, 54–60.

Atterholt, A.L. & Solensky, M.J. (2010) Effects of larval rearing density and food availability on adult size and coloration in monarch butterflies (Lepidoptera: Nymphalidae). *Journal of Entomological Science*, 45, 366–377.

Black, E.N., Blair, J.D., Van der Burg, K.R.L. & Marshall, K. (2021) Allochronic isolation on a latitudinal gradient in the polyphagous moth *Hyphantria cunea*. bioRxiv.

Caglar, S.S., Karacaoglu, C., Kuyucu, A.C. & Saglam, I.K. (2014) Humidity and seasonality drives body size patterns in males of the bush cricket *Isophya rizeensis* Sevgili, 2003 (Orthoptera: Tettigoniidae: Phaneropterinae). *Insect Science*, 21, 213–226.

Clark, K., Tremor, S., Gibson, B., Ferree, K. & Winchell, C. (2021) San Bernardino flying squirrel use of residential areas: a citizen science approach. *Western North American Naturalist*, 81, 201–206.

Davis, A.K. (2014) Intraspecific variation in wing colour is related to larval energy reserves in monarch butterflies (*Danaus plexippus*). *Physiological Entomology*, 39, 247–253.

Davis, A.K., Chi, J., Bradley, C. & Altizer, S. (2012) The redder the better: wing color predicts flight performance in monarch butterflies. *PLoS One*, 7, e41323.

Davis, A.K., Cope, N., Smith, A. & Solensky, M.J. (2007) Behaviour: wing color predicts future mating success in male monarch butterflies. *Annals of the Entomological Society of America*, 100, 339–344.

Davis, A.K., Farrey, B.D. & Altizer, S. (2005) Variation in thermally induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. *Journal of Thermal Biology*, 30, 410–421.

Douma, J.C. & Weedon, J.T. (2019) Analysing continuous proportions in ecology and evolution: a practical introduction to beta and Dirichlet regression. *Methods in Ecology and Evolution*, 10, 1412–1430.

Flockhart, D.T.T., Brower, L.P., Ramirez, M.I., Hobson, K.A., Wassenaar, L. I., Altizer, S. et al. (2017) Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. *Global Change Biology*, 23, 2565–2576.

Gautam, S. & Kunte, K. (2020) Adaptive plasticity in wing melanisation of a montane butterfly across a Himalayan elevational gradient. *Ecological Entomology*, 45, 1272–1283.

Günter, F., Beaulieu, M., Freiberg, K.F., Welzel, I., Toshkova, N., Žagar, A. et al. (2020) Genotype-environment interactions rule the response of a widespread butterfly to temperature variation. *Journal of Evolutionary Biology*, 33, 920–929.

Hanley, D., Miller, N.G., Flockhart, D.T.T. & Norris, D.R. (2013) Forewing pigmentation predicts migration distance in wild-caught migratory monarch butterflies. *Behavioral Ecology*, 24, 1108–1113.

Heidrich, L., Friess, N., Fiedler, K., Braendle, M., Hausmann, A., Brandl, R. et al. (2018) The dark side of Lepidoptera: colour lightness of geometrid moths decreases with increasing latitude. *Global Ecology and Biogeography*, 27, 407–416.

Hill, G.M., Kawahara, A.Y., Daniels, J.C., Bateman, C.C. & Scheffers, B.R. (2021) Climate change effects on animal ecology: butterflies and moths as a case study. *Biological Reviews*, 96, 2113–2126.

Jaskuła, R., Kolanowska, M. & Michalski, M. (2021) From phenology and habitat preferences to climate change: importance of citizen science in studying insect ecology in the continental scale with American red flat bark beetle, *Cucujus clavipes*, as a model species. *Insects*, 12, 369.

Kelemen, E.P. & Rehan, S.M. (2021) Opposing pressures of climate and land-use change on a native bee. *Global Change Biology*, 27, 1017–1026.

Kozlov, M.V., Oudendijk, Z., Forsman, A., Lanta, V., Barclay, M.V.L., Gusarov, I.V. et al. (2021) Climate shapes the spatiotemporal variation in color morph diversity and composition across the distribution range of *Chrysomela lapponica* leaf beetle. *Insect Science*, 29, 942–955.

Light, J.E., Keane, A.S. & Evans, J.W. (2021) Updating the distribution of American Black bears (*Ursus americanus*) in Texas using community science, state agencies, and natural history collections. *Western North American Naturalist*, 81, 396–406.

Lyons, J.I., Pierce, A.A., Barribeau, S.M., Sternberg, E.D., Mongue, A.J. & De Roode, J.C. (2012) Lack of genetic differentiation between monarch butterflies with divergent migration destinations. *Molecular Ecology*, 21, 3433–3444.

Mayekar, H. & Kodandaramaiah, U. (2021) Effect of density on pupal colour plasticity in the butterfly *Mycalesis mineus* (Lepidoptera, Nymphalidae). *Journal of Zoology*, 316, 250–262.

Mello, N.D., Sanchez, L.G.F. & Gawryszewski, F.M. (2022) Spatio-temporal colour variation of arthropods and their environment. *Evolutionary Ecology*, 36, 117–133.

Mesaglio, T., Soh, A., Kurniawidjaja, S. & Sexton, C. (2021) 'First Known Photographs of Living Specimens': the power of iNaturalist for recording rare tropical butterflies. *Journal of Insect Conservation*, 25, 905–911.

Niemiller, K.D.K., Davis, M.A. & Niemiller, M.L. (2021) Addressing 'biodiversity naivety' through project-based learning using iNaturalist. *Journal for Nature Conservation*, 64, 126070.

Nokelainen, O., Lindstedt, C. & Mappes, J. (2013) Environment-mediated morph-linked immune and life-history responses in the aposematic wood tiger moth. *Journal of Animal Ecology*, 82, 653–662.

Oberhauser, K. & Kuda, K. (1997) *A field guide to monarch caterpillars (Danaus plexippus)*. St. Paul, MN: University of Minnesota.

Polidori, C., Gutiérrez-Cánovas, C., Sánchez, E., Tormos, J., Castro, L. & Sánchez-Fernández, D. (2020) Climate change-driven body size shrinking in a social wasp. *Ecological Entomology*, 45, 130–141.

R Core Team. (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasband, W.S. (2016) ImageJ, U.S. National Institutes of Health, Bethesda, MD.

Sandre, S.L., Tammaru, T., Vanatoa, A. & Esperk, T. (2007) Maintenance of larval color polymorphism in *Orgyia antiqua* (Lepidoptera: Lymantriidae): evaluating the role of thermal adaptation. *Environmental Entomology*, 36, 1303–1309.

Solensky, M.J. & Larkin, E. (2003) Temperature-induced variation in larval coloration in *Danaus plexippus* (Lepidoptera: Nymphalidae). *Annals of the Entomological Society of America*, 96, 211–216.

Supp, S.R., Bohrer, G., Fieberg, J. & La Sorte, F.A. (2021) Estimating the movements of terrestrial animal populations using broad-scale occurrence data. *Movement Ecology*, 9, 1–19.

Talla, V., Pierce, A.A., Adams, K.L., de Man, T.J.B., Nallu, S., Villablanca, F.X. et al. (2020) Genomic evidence for gene flow between monarchs with divergent migratory phenotypes and flight performance. *Molecular Ecology*, 29, 2567–2582.

Taylor-Cox, E.D., Macgregor, C.J., Corthine, A., Hill, J.K., Hodgson, J.A. & Saccheri, I.J. (2020) Wing morphological responses to latitude and colonisation in a range expanding butterfly. *PeerJ*, 8, e10352.

True, J.R. (2003) Insect melanism: the molecules matter. *Trends in Ecology and Evolution*, 18, 640–647.

Tseng, M., Kaur, K.M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C.H. et al. (2018) Decreases in beetle body size linked to climate change and warming temperatures. *Journal of Animal Ecology*, 87, 647–659.

Välimäki, P., Kivelä, S.M., Raitanen, J., Pakanen, V.-M., Vatka, E., Mäenpää, M.I. et al. (2015) Larval melanism in a geometrid moth: promoted neither by a thermal nor seasonal adaptation but desiccating environments. *Journal of Animal Ecology*, 84, 817–828.

Wang, T., Hamann, A., Spittlehouse, D. & Carroll, C. (2016) Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS One*, 11, 1–17.

Willadsen, P.C. (2022) Aculeate hymenopterans as aposematic and mimetic models. *Frontiers in Ecology and Evolution*, 10, 827319.

Yamanaka, A., Tanaka, A. & Kitazawa, C. (2012) Pupal color polyphenism regulated by temperature and photoperiod in the Asian comma butterfly, *Polygonia c-aureum* (Lepidoptera: Nymphalidae). *Zoological Studies*, 51, 1432–1437.

Young, B.E., Lee, M.T., Frey, M., Barnes, K. & Hopkins, P. (2021) Using citizen science observations to develop managed area watch lists. *Natural Areas Journal*, 41, 307–314.

Yumnam, T., Banerjee, B. & Kodandaramaiah, U. (2021) Pupal colour plasticity in the butterfly *Catopsilia pomona* (Lepidoptera: Pieridae). *Biological Journal of the Linnean Society*, 134, 331–341.

SUPPORTING INFORMATION

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

Figure S1. When searching for monarch larvae images on iNaturalist, we ignored photos where the black stripes were not distinct. Below is an example of a larva with fuzzy black stripes. Photo credit: faulcon, CC-BY-NC (INaturliast ID: 88168346)

Figure S2. As requested by a reviewer we have included the Q–Q plots for (a) the raw data; (b) the model of Caterpillar Percent Black ~ Average Summer Temperature + Photo view; and (c) for the model of Caterpillar Percent Black ~ Latitude × Year + Photo view.

Table S1. Cities used to quantify summer temperature in the study region.

How to cite this article: Tseng, M., Bevanda, C., Bhatti, S.S., Black, E.N., Chang, E., Chiang, J. et al. (2022) Effects of temperature on monarch caterpillar pigment variation in nature. *Insect Conservation and Diversity*, 1–8. Available from: https://doi.org/10.1111/icad.12608