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Movement Ecology

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Effects of temperature experienced across life stages on morphology and flight behavior of painted lady butterflies (*Vanessa cardui*)

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Abstract

Background With ongoing anthropogenic climate change, there is increasing interest in how organisms are affected by higher temperatures, including how animals respond behaviorally to increasing temperatures. Movement behavior is especially relevant, as the ability of a species to shift its range is implicitly dependent upon movement capacity and motivation. Temperature may influence movement behavior of ectotherms both directly, through an increase in body temperature, and indirectly, through temperature-dependent effects on physiological and morphological traits.

Methods We investigated the influence of ambient temperature during two life stages, larval and adult, on body size and movement behavior of the painted lady butterfly (*Vanessa cardui*). We reared painted ladies to emergence at either a "low" (24 °C) or "high" (28 °C) temperature. At eclosion, we assessed flight behavior in an arena test. We used a full factorial experimental design in which half of the adults that emerged from each rearing treatment were tested at either the "low" or "high" temperature. We measured adult body size, including wingspan, and determined flight speed, distance, and duration from video recordings.

Results Adult butterflies that experienced the higher temperature during development were larger. We documented an interaction of rearing x testing temperature on flight behavior: unexpectedly, the fastest butterflies were those who experienced a change in temperature, whether an increase or decrease, between rearing and testing. Individuals that experienced matching thermal environments flew more slowly, but for more time and covering more distance. We found no influence of body size per se on flight.

Conclusions We conclude that the potential role of "matching" thermal environments across life stages has been underinvestigated with regard to how organisms may respond to warming conditions.

Keywords Climate change, Temperature, Flight, Movement behavior, Butterfly

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Introduction

Anthropogenic climate change continues to influence life on Earth [1, 2], with diverse responses to increasing temperatures exhibited by a range of species. Potential responses include adaptive evolution, plastic acclimation to new conditions, movement in either space (range shifts, typically to areas of higher latitude and/or elevation) or time (phenological shifts), and even the most extreme response: extinction. However, while predictions about future range shifts in relation to increasing temperatures are common in the literature, the movement behavior that would underlie such shifts by animal species is not often considered [3–6]. Many factors can influence an organism's ability to move from one location to another and the search for more resources can be impacted by external conditions, including temperature [7].

Ambient temperature can influence movement behavior in multiple ways, including both direct and indirect effects. For ectothermic animals in particular, the rate of vital processes is affected by thermal conditions [8]. In many cases, the thermal performance curve is "hump-shaped": performance (or the rate of a process) increases with increasing temperature up to the thermal optimum, then declines [9]. We might expect the locomotion speed of an ectothermic animal to follow such a performance curve, in which speed increases with temperature to the thermal optimum, then declines dramatically [6].

However, temperature can also have indirect effects on movement behavior. For example, thermal influences on developmental processes may produce variation in movement-related morphology [3]. Body size is a well-known predictor of movement behavior in a range of animal species [10]. Changes in body size are also a major response to warmer temperatures [11, 12], with the "temperature-size rule" stating that organisms should develop faster and be smaller at higher temperatures, leading some to predict that body sizes will decline with increasing temperatures due to anthropogenic climate change [13]. Thus, body size becomes a potential explicit link between the effect of climate warming on organisms and the ability of those organisms to respond to warming via movement [3]: animals that develop under warmer conditions are expected to be smaller as adults, and thus potentially less able to track changing environmental conditions.

Despite the importance of movement behavior as an influence on range shifts in response to climate change, relatively few have attempted to link the developmental conditions that produce variation in movement-related phenotypes to movement behavior later in life. This is potentially related to a mismatch in the types of studies and systems used to investigate these questions: there has been a recent explosion in techniques for tracking the movements of large animals in the field [14–16], but it is almost impossible to manipulate rearing conditions for these species in ways that should result in morphological changes relevant to movement. Meanwhile, it is relatively straightforward to generate such morphological diversity in lab-based invertebrate systems, but devices for tracking individual movements of very small animals are more limited and our understanding of thermal effects on movement of insects remains incomplete [6, 17, 18]. However, it is often feasible to track the movements of individuals using automated systems in the laboratory [6], and this is the approach that we followed in this study.

To understand the effects of temperature on both body size and movement behavior, we used the painted lady butterfly (*Vanessa cardui*) as a study organism. In general, butterflies serve as pollinators, food sources and indicators of ecosystem health [19], and butterfly range shifts have been documented in relation to climate change [20]. *V. cardui* is particularly suitable for the study of movement behavior due to its almost cosmopolitan distribution and high mobility [21]; *V. cardui* exhibits mass migratory behavior [22], and transatlantic flights have recently been reported [23]. We manipulated rearing temperature for larval *V. cardui* and flight testing temperature for adults in a two-way factorial design. First, we wanted to determine how rearing temperature influenced adult morphology. Specifically, we were interested in effects of temperature on body size, due to its potential influence on movement behavior [3]. Following the "temperature size rule" [11], we predicted that at emergence, butterflies reared at a higher temperature would be smaller than those reared at a lower temperature. Second, we wanted to determine the influence of ambient temperature on flight behavior. Because we predicted that larger adult butterflies (presumably those reared at a lower temperature) would fly farther and faster than would smaller individuals, we needed to control for potential effects of variation in the rearing environment on flight-related morphology in adults, which we did using a fully factorial experimental design. Combining our predictions about the effects of rearing temperature on body size ("hotter is smaller") and testing temperature on flight ("hotter is faster"), we expected that individuals who experienced lower rearing temperatures and higher flight testing temperatures would fly the fastest and in contrast, animals that were reared at a higher temperature and tested at a lower temperature would be the slowest.

Methods

Study species and culturing

We obtained larval *V. cardui* from Carolina Biological Supply (Burlington, NC, USA). Larvae were within one developmental instar from each other upon receipt.

Three randomly-selected larvae were placed into each rearing container without consideration of size or stage of development. With a paintbrush, larvae were gently transferred from travel containers to prepared rearing containers, each of which contained approximately 30 g of food mix in an even layer on the bottom of the cup (painted lady culture medium, Carolina Biological Supply, Burlington, NC, USA). Each container was then covered with a mesh screen to allow for hanging chrysalids. A second plastic lid with air holes was placed on the cup to prevent larvae from escaping. Food was replenished as it was consumed and detritus was removed from rearing containers.

Manipulation of developmental temperatures

Experimental temperature regimes were based on established temperature tolerances of *V. cardui* in laboratory studies. Symanski and Redak [24] reviewed rearing temperatures in laboratory studies of *V. cardui*; they report experiments across a range from 12 to 33 °C, with an increase in mortality at temperatures above 30 °C, and describe 25 °C as the "optimal" rearing temperature. Our objective was to induce deleterious effects without increasing mortality, so we chose 24 °C ("low") and 28 °C ("high") as our temperature treatments. We used an environmental chamber with two separate chambers that were individually programmed to maintain the experimental rearing temperature (BioChambers model #6194, Winnipeg, Manitoba, Canada). This chamber maintains a temperature ± 0.5 °C of the set temperature, per manufacturer specifications. Lights came on at 0800 and went off at 2000 daily. Each larval container was randomly assigned to one of the rearing temperatures.

Larvae were monitored daily from 21 October 2022 to 8 December 2022, at which point all larvae had either metamorphosed or died. We determined the number of live and dead larvae, chrysalides, and newly emerged adults in each container each day. There was some evidence of cannibalism: half bodied larvae and larvae parts including decapitated heads were observed prior to the chrysalid form, when larval heads are pinched off. Because we housed 3 larvae/cup, we could not determine which individual was responsible for cannibalism.

Once an individual reached chrysalis form, it was moved from the rearing container and placed into a 42 cm x 42 cm x 76 cm enclosure constructed of 1.27 cm diameter PVC pipe and mesh netting, with one enclosure per temperature treatment. These enclosures were kept in the environmental chambers with the remaining rearing containers. A zippered door and Velcro corner allowed for the handling of chrysalides and adults. Chrysalides were hung within the mesh containers along the walls to allow for proper development of wings after eclosion. Once an adult eclosed it was assigned a color which was painted on the dorsal side of the abdomen with non-toxic hobby paint (Apple Barrel acrylic paint, PLAID, Atlanta, GA, USA) and allowed for the simultaneous tracking of multiple individuals in filmed flight trials. Food for adults was a 1:4 mixture of sucrose and water which was placed in the adult enclosures in a tray with a sponge on top of the liquid to help prevent drowning.

Measuring movement behavior under varying temperatures

The movements of adult butterflies were assessed at both low (24 °C) and high (28 °C) temperatures. Because we expected movement to be affected by both temperature during the flight tests [25] and the temperature at which they were reared (via an effect of rearing temperature on body size), we used a two-way factorial design in the assessment of movement behavior. Animals that were reared under the low and high temperature regimes were split evenly between testing temperatures for a total of four treatments: low-low, high-high, low-high, high-low, where "low" = 24 °C and "high" = 28 °C. Flight tests were conducted in an enclosed space that was warmed to the required testing temperature using a space heater (Dreo Atom One Space Heater, Dreo, New York, NY, USA), under standard room lighting. Temperature was confirmed using a thermometer. The flight arena was a 92 cm x 43 cm x 46 cm aquarium (Aqueon standard open-glass aquarium tank, 151.4 L, Aqueon Products, Franklin, WI, USA) with a fitted lid to prevent escape of individuals during trials. The glass walls of the aquarium allowed video recording and prevented individuals from clinging to the side rather than flying.

For each flight trial, five randomly selected marked adults were placed in the arena. Each trial lasted for one hour and was recorded using a small video camera (Akaso V50 X action camera, Akaso, Frederick, MD, USA). The lights were on for the entirety of the trials. After the flight trial, the butterflies were placed in another mesh enclosure of the same size (42 cm x 42 cm x 76 cm) instead of the general population enclosure so they would not be selected again.

Morphological measurements

After flight trials, adults were placed into envelopes with wings folded to prevent damage and secured in a -20 °C freezer. Each envelope was marked with an individual's color, flight trial number, and unique ID number. Individuals were later pinned, spread, and dried. After drying for two days, we measured total wingspan, body length, and forewing length (all in mm) using electronic calipers (15.5 cm electronic digital caliper w/ LCD readout, WEN, West Dundee, IL, USA). We did not determine sex of tested animals; sex size dimorphism is reported to be minimal in this species [26].

Scoring of movement behavior

To allow butterflies to acclimate to the testing environment, the first 15 min of each flight trial were discarded. Flight behaviors over the next 30 min were scored using idTracker software [27], which allowed us to simultaneously track multiple individual flight paths. For each individual, we quantified total time spent in the air (s) and total distance traveled (cm). Speed (cm/s) was calculated from flight time and travel distance measurements.

Statistical analysis

All statistical analyses were conducted in R version 4.2.3 [28]. Due to expected correlations among body size measurements, we first tested for statistical correlations among wingspan, body length, and forewing length. We then tested for an effect of rearing temperature treatment on body size using a t-test. To determine the effects of experimental treatments on movement, we used separate statistical models for each of three responses: speed (cm/s), time in air (s), and distance moved (cm). Each model included main effects of rearing temperature and testing temperature, the interaction of the two temperature treatments, and a covariate: wingspan, which accounted for the possibility that larger animals were stronger flyers. Further, for each response variable, we ran models with and without a random effect of flight trial to account for potential non-independence among individuals who were tested together. All models were run with the R package ‘lmerTest’ [29] using Type III sums of squares. Finally, we compared the performance of models with and without the random effect of flight trial using AICc values and model weights generated using the package ‘performance’ [30], and report results from the higher-ranking model for each response variable.

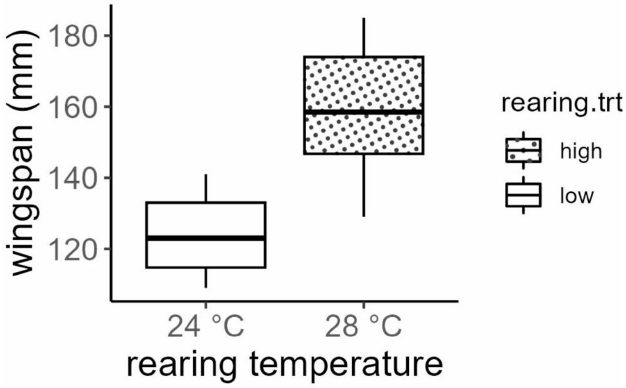

Fig. 1 Boxplot showing the influence of rearing temperature on adult *V. cardui* wingspan. The open bar shows wingspan for animals reared at 24 °C and the stippled bar shows animals reared at 28 °C

Results

A total of 398 adult butterflies emerged from the rearing treatments (N: low=206, high=192), and there was no statistically significant effect of rearing temperature on survival (χ^2=0.18, P=0.67). As expected, all body size measurements were highly correlated with each other (wingspan to body length: r=0.66, wingspan to forewing length: r=0.71, body length to forewing length: r=0.75). Thus, we chose a single measurement, wingspan, to assess the morphological response to rearing temperature. In contrast to our expectation based on the temperature-size rule, high rearing temperature individuals had larger wingspans than did low rearing temperature individuals (mean ± 1 SE (mm): low=124.12 ± 0.69, high=159.17 ± 1.13; Fig. 1, t=169.13, $P<2\times10^{-16}$).

We found statistically significant interaction effects of rearing x testing temperature on all movement responses. The addition of a random effect of flight trial did not substantially improve performance of any model (speed: DAICc=2.1, model weight=0.73; time: DAICc=2.1, model weight=0.74; distance: DAICc=1.1, model weight=0.64; in all cases, the model without the random effect had the lower AICc value), thus we proceeded with simpler GLMs. For flight speed, butterflies in the low/low and high/high treatments flew significantly slower than did the individuals who experienced different rearing and testing treatment temperatures (GLM: rearing temperature, $F_{1,\,393}$ = 8.55, P=0.004, testing temperature, $F_{1,\,393}$ = 30.08, $P=7.44\times10^{-08}$, interaction, $F_{1,\,393}$ = 113.28, $P<2.2\times10^{-16}$, wingspan, $F_{1,\,393}$ = 0.003, P=0.96; mean ± 1 SE (cm/s): low/low: 3.61 ± 0.06, high/high: 4.08 ± 0.19, low/high: 7.01 ± 0.31, high/low: 5.27 ± 0.21; Fig. 2). When we decomposed speed into its two components (time flying and distance traveled), we also observed statistically significant interaction effects, and all treatment combinations were different from each other (Figs. 3 and 4). However, butterflies from the low/low and high/high treatments flew longer (GLM: rearing temperature, $F_{1,\,393}$ = 0.06, P=0.81, testing temperature, $F_{1,\,393}$ = 25.86, $P=5.69\times10^{-07}$, interaction, $F_{1,\,393}$ = 379.68, $P<2.2\times10^{-16}$, wingspan, $F_{1,\,393}$ = 1.87, P=0.17; mean ± 1 SE (seconds): low/low: 1,166.85 ± 19.54, high/high: 1,058.25 ± 38.41, low/high: 542.04 ± 20.35, high/low: 663.50 ± 23.50; Fig. 3) and for greater distances (GLM: rearing temperature, $F_{1,\,393}$ = 154.94, $P<2.2\times10^{-16}$, testing temperature, $F_{1,\,393}$ = 57.19, $P=2.83\times10^{-13}$, interaction, $F_{1,\,393}$ = 1262.50, $P<2.2\times10^{-16}$, wingspan, $F_{1,\,393}$ = 0.005, P=0.94; mean ± 1 SE (cm); low/low: 4,093.00 ± 14.53, high/high: 3,689.48 ± 32.87, low/high: 3,192.82 ± 17.24, high/low: 3,060.72 ± 17.79; Fig. 4) than individuals in the other treatments. Thus, butterflies that experienced matching rearing and testing temperatures, whether low or high, flew further and for longer than animals from the other treatments (Figs. 3 and 4), but at lower speeds (Fig. 2).

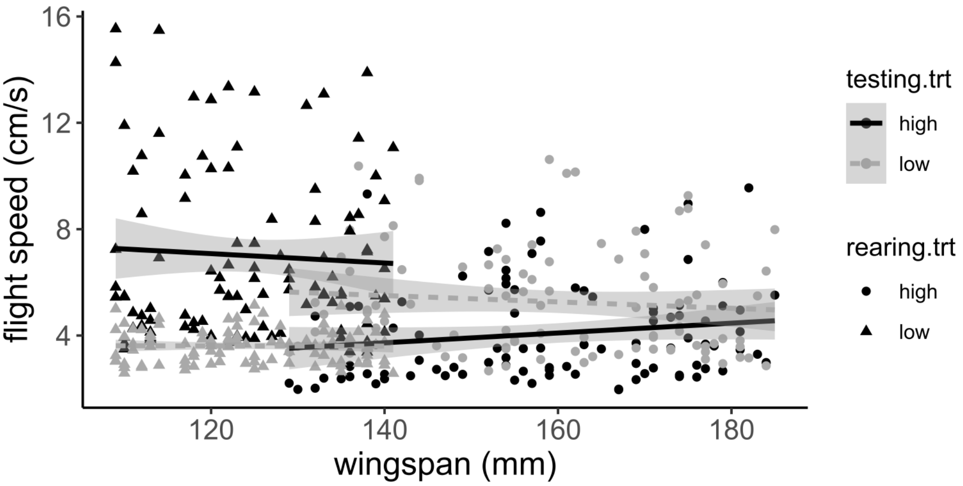

Fig. 2 Scatterplot showing the effects of rearing temperature, flight testing temperature, and wingspan on flight speed (cm/s). Different symbols represent the rearing temperatures (triangles = 24 °C, circles = 28 °C). Gray symbols and dashed lines represent the low (24 °C) testing temperature and black symbols and solid lines represent the high (28 °C) testing temperature. Best-fit lines show the linear model speed ~ wingspan for each rearing x testing temperature combination. The shaded area depicts the 95% confidence interval

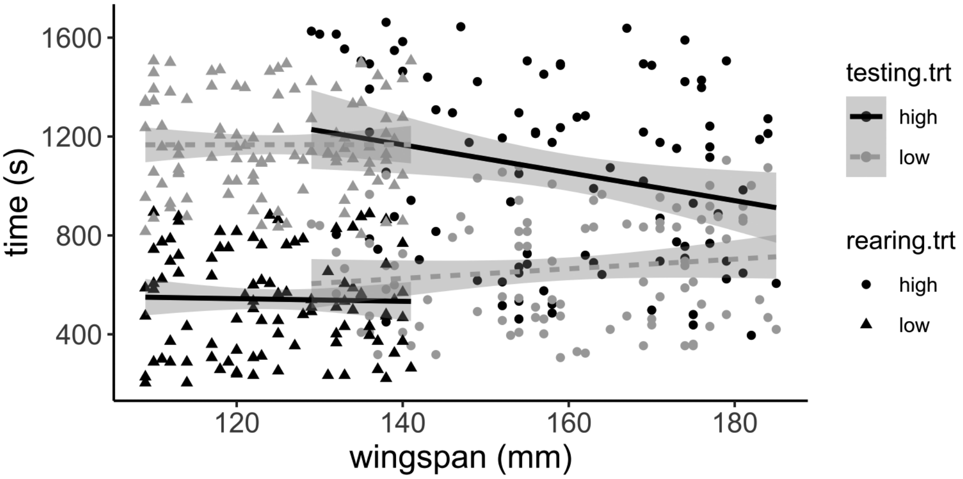

Fig. 3 Scatterplot showing the effects of rearing temperature, flight testing temperature, and wingspan on time spent flying (s). Different symbols represent the rearing temperatures (triangles = 24 °C, circles = 28 °C). Gray symbols and dashed lines represent the low (24 °C) testing temperature and black symbols and solid lines represent the high (28 °C) testing temperature. Best-fit lines show the linear model time flying ~ wingspan for each rearing x testing temperature combination. The shaded area depicts the 95% confidence interval

The covariate "wingspan" was not a statistically significant predictor of any movement response.

Discussion

We found a series of unexpected results in our investigation of the effects of rearing and testing temperatures on flight behavior by *V. cardui*. The first surprising result, that a higher rearing temperature resulted in larger rather than smaller adult body size (Fig. 1), fundamentally changed our expectations for the rest of the experiment. Given that larger animals were produced by the higher rearing temperature, we now expect that the lowest flight speeds would be exhibited by low/low treatment animals (smaller bodies at lower temperatures), and the highest flight speeds by high/high individuals (larger bodies at higher temperatures). However, our observations did not align with these revised predictions, either. Instead, we found that higher flight speeds were exhibited by the animals who experienced a mismatch between the conditions under which they were reared and those under which they were tested (Fig. 2). Further, we found no effect of wingspan (i.e., body size) on any aspect of flight behavior.

Our first unexpected result was the increase in body size (quantified as wingspan) with higher rearing temperatures (Fig. 1). However, experimental rearing studies increasingly suggest that the temperature size rule is less consistent for arthropods than for vertebrate

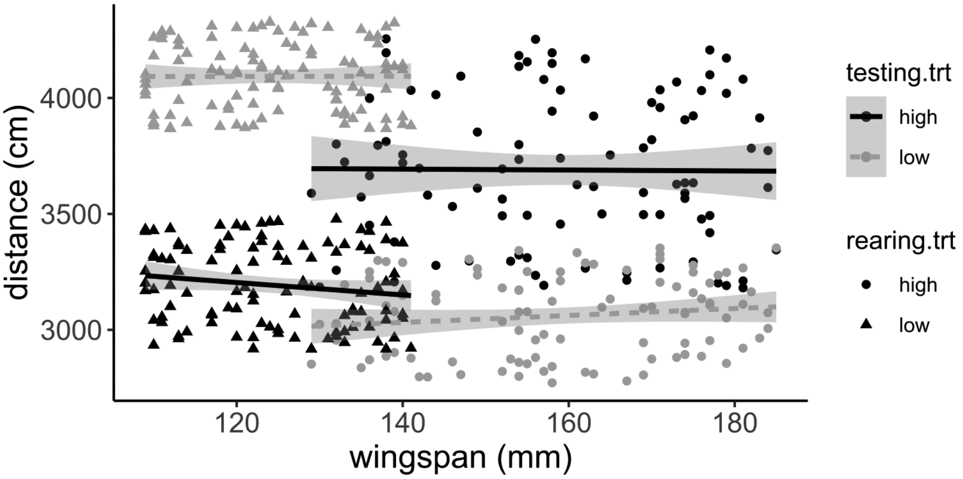

Fig. 4 Scatterplot showing the effects of rearing temperature, flight testing temperature, and wingspan on flight distance (cm). Different symbols represent the rearing temperatures (triangles = 24 °C, circles = 28 °C). Gray symbols and dashed lines represent the low (24 °C) testing temperature and black symbols and solid lines represent the high (28 °C) testing temperature. Best-fit lines show the linear model flight distance ~ wingspan for each rearing x testing temperature combination. The shaded area depicts the 95% confidence interval

ectotherms [31–33], with some studies even describing a "reverse TSR" [34], in which size increases with temperature [5, 32]. Further, recent meta-analyses have failed to find evidence of selection for smaller bodies at higher temperatures [35, 36] (note that these studies focus on evolutionary rather than plastic responses to temperature). Published reports of effects of rearing temperature on body size in *V. cardui* are sparse, but as in the current study, the data collected by Medina-Báez and colleagues [27, 37] are not consistent with the TSR. Although Medina-Báez et al. (2023) did not directly report the effect of rearing temperature on body size, we were able to assess this using their archived data [37], which showed that *V. cardui* reared at 20 °C and 30 °C were not significantly different in body mass (mg) as adults. The range of observed responses of insect body sizes to experimental warming treatments suggests that McCauley and Mabry's [3] concept of a positive feedback loop between temperature and body size that negatively affects movement is perhaps more broadly applicable to vertebrate than invertebrate ectotherms.

Our second surprising result was the lack of effect of wingspan on movement behavior (Figs. 2, 3 and 4). We did not find that larger butterflies were more mobile; instead, the main influence on flight behavior was temperature. Animals that experienced "matching" rearing and testing environments flew longer and covered more distance (Figs. 3 and 4), at lower flight speeds (Fig. 2). We posit that this observation constitutes a carry-over effect, in which environmental conditions experienced early in life affect animals at a later life stage [38], potentially via developmental influences on physiology and behavior. We are not alone in observing acclimation effects to rearing temperature in *V. cardui*; albeit with a different response, Medina-Báez et al. [26] found that individuals reared at 30 °C had a higher critical thermal maximum (CT_{max}) than did butterflies reared at 20 °C, and that CT_{max} also varied across ontogeny.

Our result has potential implications not only for understanding how insects may respond to climate change, but also for the design of experiments seeking to investigate movement as a response to temperature across life stages. While we deliberately set out to control for potential carry-over effects in understanding how temperature and body size influenced the flight behavior of *V. cardui*, many studies of temperature effects on animals across ontogeny do not conduct such controls (as reviewed by [39]). In studies of thermal effects on movement of invertebrates, it is not uncommon for researchers to rear animals at different temperatures, but conduct all movement tests at the same temperature [17, 40]. Alternately, some researchers use a single rearing temperature and multiple testing temperatures [6]. Both types of experimental design can confound acclimation to a temperature and carry-over effects. This is because when such experiments use multiple temperatures for either rearing or testing (but not both), alignment of rearing and testing conditions typically occurs at just one temperature (the control), and it is not possible to evaluate whether animals reared at other temperatures would demonstrate similar responses if movement were tested at their respective rearing temperature. For example, Arambourou et al. [40] observed reduced flight time in damselflies reared under "heat wave" conditions and conclude that "carry-over effects of warming experienced during the larval stage reduce adult locomotor ability". However, because their damselflies were reared at three different temperatures (20, 25, and 30 °C) and flight trials were conducted at one of those temperatures (20 °C), this finding is consistent with both carry-over effects and the degree of acclimation to the temperature under which flight was assessed. Put more simply, "heat wave" damselflies experienced elevated temperatures during development, but also experienced a dramatic decrease in temperature (of 5–10 °C) when movement behavior was assessed. Experiments in which both rearing and testing temperatures are manipulated will always be logistically challenging, if only because of limited availability of environmental chambers. However, we argue that given the potential influence of both thermal acclimation and carry-over effects on movement behavior of ectotherms in particular, it is imperative to use fully-replicated experimental designs or employ a gradient experimental design [41].

Multiple aspects of morphology have been shown to affect flight performance in butterflies: for example, Barwaerts et al. [42] found effects of body mass, thorax mass, forewing area, forewing length, wing loading, aspect ratio, and the centroid of forewing area on flight performance. Barwaerts et al. [42] found that all of the listed traits correlated positively with flight performance. Understandably, however, most researchers (including us) do not measure all of these variables when assessing the effects of flight morphology on flight performance and behavior. Differences in which variables were measured among studies complicate efforts at synthesis, as traits deemed important in one study may be unmeasured in others. For example, Reim et al. [17] found that increased rearing temperature did lead to smaller body size in the tropical butterfly *Bicyclus anynana*, but also found that sexual dimorphism was a more important influence than rearing temperature on flight behavior. Reim and colleagues [17] reared their butterflies at three temperatures (21, 25, and 29 °C) and conducted flight tests at 27 °C, finding that flight distance was shorter at higher temperatures. The closest analog to the results of Reim et al. [17] in the current study is the comparison between groups of *V. cardui* that were reared at either 24–28 °C and given flight tests at 28 °C (Fig. 4). In contrast to their findings, we documented increased flight distance by butterflies reared at the higher temperature. However, we also documented smaller body sizes for *V. cardui* reared at the lower temperature, the opposite of the findings for *B. anynana*, making it difficult to directly compare the two studies in terms of the effects of rearing temperature on movement behavior.

Conclusions

Drawing synthetic conclusions about how the movement behavior of insects may respond to climate change is challenging. As described above, differences in experimental designs across studies make comparisons difficult, as researchers vary in both how they manipulate rearing and testing temperatures and in the response variables measured. Further, recent studies have found unexpected results – for example, Arambourou et al. [40] found that increased rearing temperature decreased flight performance in a damselfly, but via a change in wing shape rather than body size. Thus, while insight into how thermal conditions influence development and subsequent movement behavior is needed to make realistic predictions about future changes [4, 17, 43–45], organismal responses may be complex and potentially unexpected [17, 32].

Supplementary Information

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Supplementary Material 1

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Author contributions

Both authors contributed to conception, study design, and data interpretation. SPM conducted all experimental work and wrote the first draft of the manuscript. KEM contributed to revisions and prepared the figures. Both authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

No permits or approvals were required for the experiments with invertebrate animals reported here.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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