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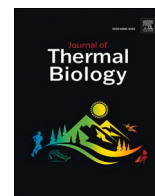
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# Exposure to elevated temperature during development affects eclosion and morphology in the temperate *Pieris napi* butterfly (Lepidoptera: Pieridae)

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## ABSTRACT

Global warming has been identified as one of the main drivers of population decline in insect pollinators. One aspect of the insect life cycle that would be particularly sensitive to elevated temperatures is the developmental transition from larva to adult. Temperature-induced modifications to the development of body parts and sensory organs likely have functional consequences for adult behaviour. To date, we have little knowledge about the effect of sub-optimal temperature on the development and functional morphology of different body parts, particularly sensory organs, in ectothermic solitary pollinators such as butterflies. To address this knowledge gap, we exposed the pupae of the butterfly *Pieris napi* to either 23 °C or 32 °C and measured the subsequent effects on eclosion, body size and the development of the wings, proboscis, eyes and antennae. In comparison to individuals that developed at 23 °C, we found that exposure to 32 °C during the pupal stage increased mortality and decreased time to eclose. Furthermore, both female and male butterflies that developed at 32 °C were smaller and had shorter proboscides, while males had shorter antennae. In contrast, we found no significant effect of rearing temperature on wing and eye size or wing deformity. Our findings suggest that increasing global temperatures and its corresponding co-stressors, such as humidity, will impact the survival of butterflies by impairing eclosion and the proper development of body and sensory organs.

## 1. Introduction

Insect pollinators play a key role in biodiversity and ecosystem maintenance but their populations have undergone an alarming decline in recent decades due to anthropogenic activities (Kerr et al., 2015; Agrawal and Inamine, 2018; Zattara and Aizen, 2021). While global warming has been identified as one of the main drivers of this decline (Soroye et al., 2020), the underlying mechanisms remain unclear (Dicks et al., 2021). One aspect of the insect life cycle that could be particularly sensitive to elevated temperatures is the developmental transition from larva to adult. This is primarily because the development rate and final size of body parts can be affected differently by variations in ambient temperature (Shingleton et al., 2009) due to differences in the thermal sensitivity of the tissue that gives rise to them (French et al., 1998). In *Drosophila melanogaster*, for example, temperature shifts lead to differences in the growth rate of the wings and the thorax (French et al., 1998; McDonald et al., 2018).

Understanding how the development of different body parts is affected by elevated temperature in insect pollinators could be particularly important for predicting the effects that current and future rapid climate changes will have on the ecosystem services they provide. This is because any potential disruptions to the development of the body, wings and sensory organs due to sub-optimal temperature would likely impair the function of these organs and therefore reduce insect pollinator ability to not only fly between and locate floral resources but also to mate and reproduce. In one of only two studies on the effect of elevated developmental temperatures on several body parts of adult insect pollinators, Perl et al. (2022) found that exposure to elevated temperature during the final period of adult development did not affect body size, wing size or sensory system development in bumblebees. However, this exposure did affect their ability to respond appropriately to sensory stimuli, suggesting that their neural function had been impaired. A later study that exposed bumblebees to elevated temperatures during the entire pupa-adult developmental period found that workers had reduced

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body sizes and that both workers and males had shorter antennae when compared to individuals reared under optimal conditions but wing size was not affected (Gérard et al., 2023). It is important to note that these studies focussed on the effect of developmental temperature on bumblebees that, being eusocial insects, can mitigate the effects of increasing environmental temperatures by tightly regulating the temperature of the developing brood (Gardner et al., 2007). In contrast, ectothermic solitary pollinators, such as butterflies, are directly exposed to environmental temperature fluctuations during the pupal development stage. Exposure to elevated temperature during development has been reported to increase mortality rate and decrease body size and development rate in early and also pupal stage in many groups of ectothermic insects, such as ants (Molet et al., 2017), moths (Tamiru et al., 2012) and butterflies (Stuhldreher et al., 2014; Huang et al., 2022). However, how the sensory morphology of Ectothermic insects is affected by exposure to elevated temperatures during development and the functional consequences of these changes remains unclear.

To address this knowledge gap, we investigated the effect of elevated temperature on the pupal development and adult morphology of the butterfly *Pieris napi* (Lepidoptera: Pieridae). This butterfly is a habitat generalist and is an important pollinator in temperate regions, particularly in Northern European countries such as Sweden. Female and male of *P. napi* feed on a broad range of flowers (Goulson and Cory, 1993; Corbet, 2000). Pupation usually occurs at the host plant or on nearby vertical surfaces 2–3 weeks prior to adult eclosion (Chinery, 1998). In recent years, populations of *P. napi* in Sweden have been exposed to temperatures in excess of those they normally experience during the pupal development. To explore how such changes in climatic conditions may affect *P. napi* butterflies, we exposed their pupae to temperatures of either 23 °C or 32 °C throughout the duration of their development and assessed the subsequent effects on their eclosion and development of body and sensory organs. The lower experimental temperature of 23 °C was chosen because it lies within the range of temperatures in summer that *P. napi* pupae go under direct development (Larsdotter-Mellström and Wiklund, 2015), while the elevated temperature of 32 °C was chosen as this is the upper limit at which this species survives during the pupal stage (von Schmalensee et al., 2023). Furthermore, these experimental temperatures range from those that are frequently experienced (e.g. 23 °C) to those that will become more common (e.g. 32 °C) during summer at the site where the specimens used in this study were collected (see Appendix A, Figure S1, Table S1).

## 2. Materials and methods

### 2.1. Study organism

Wild male and female *Pieris napi* (Lepidoptera: Pieridae) were collected daily (09:00–18:00) in late August 2019 in Ransvik, southern Sweden (56°17'3" N, 12°29'50" E). The average ambient temperature for this month was 21 °C (The Swedish Agency for Marine and Water Management and SMHI, Helsingborg station). The butterflies were moved to a laboratory at Stockholm University, where they were kept in cages (0.8 m × 0.8 m × 0.5 m) for breeding. The cages were illuminated with 400 W HQIL lamps from 09:00–17:00 (8 h photophase) with a temperature of 28 °C during the photophase and 20 °C during the scotophase. The butterflies were fed *ad libitum* with 20% sugar solution droplets on *Kalanchoe* sp. flowers and were provided with *Alliaria petiolata* (one of the main host plants of this species) leaves for egg-laying. Leaves with eggs from the F2 generation were collected in November 2019. The specimens were placed in a rearing room at 23 °C with 22:2 h light:dark cycle and 100% humidity until they hatched. Under these conditions, *P. napi* from temperate regions undergo direct development from egg to adult, without a pupal diapause. Newly hatched larvae were moved to plastic containers (3–4 larvae per container) filled with 100 ml water (to maintain humidity) and covered with four leaves of *Alliaria petiolata*. A total of 120 larvae were randomly

distributed among the containers and were kept in the rearing room until the first stage of chrysalis formation. Larval status was checked daily, and dried leaves were continually replaced with fresh leaves.

### 2.2. Mortality and pupal development

Newly pupated specimens were collected daily, sexed under a stereoscope and transferred to individual cups that were sealed with a fabric net and lined with paper to facilitate movement after eclosion. Each cup was placed in one of the two climate chambers kept at either 23 °C or 32 °C (Fig. 1A). The climate chambers had a 22:2 h light: dark cycle. A climate logger (EasyLog, EL-USB-2, Lascar Ltd., UK) was placed in each chamber to record temperature and humidity. The data from the climate loggers showed that the temperature was maintained throughout the experiment but that the relative humidity was 30% in the 23 °C chamber and 20% in the 32 °C chamber due to minor variations between the systems controlling each one. Mortality rate was assessed after the last viable individuals were fully eclosed (Steigenga and Fischer, 2009) and was defined as the number of individuals that did not eclose (after four weeks, as normal development takes 2–3 weeks (Chinery, 1998), or that died (dried out and/or became hollow) during (Fig. 1B), or immediately after, eclosion. Pupal development time for each temperature treatment was defined as the number of days between chrysalis formation and adult eclosion. Specimens used for subsequent morphometric measurements were euthanised 12 h after eclosion (to ensure that each individual had fully completed development) by exposing them to ethyl acetate for 15 min.

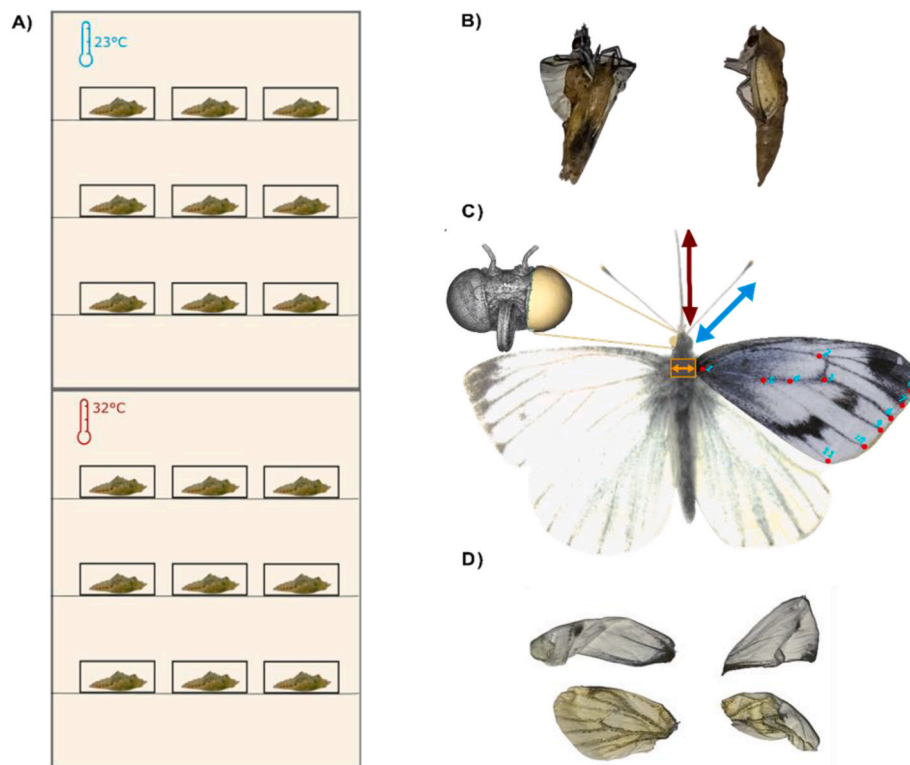
### 2.3. Morphometric measurements

#### 2.3.1. Sample preparation

To measure body size, the whole body was laid flat on a plain paper next to a 1 mm black square and photographed. The right forewing, uncoiled proboscis and left antenna were dissected, laid flat on 1 mm grid paper and photographed for measurements. If the wing was slightly rolled up at the tip or slightly wrinkled, it was flattened carefully and taped to the paper. The left half of the head (including the compound eye) was dissected and placed in 75% ethanol before being stained with 0.5% phosphotungstic acid (PTA), dehydrated in an ethanol series and embedded in epoxy resin, following the methods described in Taylor et al. (2019). The head samples were scanned at the TOMCAT beamline of the Swiss Light Source, Paul Scherrer Institute, Villigen (Switzerland) (beamtime number 20190641) with monochromatic X-ray beam set to 20 keV energy. Each scan was taken at 4× total magnification and consisted of 2001 projection images that had 2569 × 2160 pix and a 100 ms exposure time. The resulting images were reconstructed into 3D volumes using the gridrec algorithm with Parzen filter, resulting in 16-bit volume images with an isotropic voxel size of 1.6 µm.

#### 2.3.2. Sample measurement

Thorax width was calculated by finding the length of the short axis of a bounding box fitted around the body (Fig. 1C) using an in-house automated program. This length was converted from pix to mm values using a 1 mm × 1 mm reference square located in the same image. Wing size and shape were examined with geometric morphometric analysis using landmarks (Rohlf et al., 1996; Adams et al., 2004), following the procedure described in Bai et al. (2015). A total of 11 homologous landmarks were selected on the vein intersections and terminations of the forewing of each specimen and digitised (Fig. 1C) using TpsDig.2.32 (Rohlf, 2015). Wing size was measured using centroid size, i.e. the square root of the sum of squared distance between all landmarks and their centroid (Zelditch et al., 2012). Forewings with major deformities – defined by not being possible to flatten them or to identify landmarks for the geometric morphometric analysis (Fig. 1D) – were excluded from the wing analysis and were included in the wing deformity analysis instead. Proboscis length and antennal stalk length (Fig. 1C) were scaled using a



**Fig. 1.** A) Graphical illustration of the climate chambers where *Pieris napi* pupae were reared in two temperature groups (23 °C and 32 °C) in individual cups (rectangles), B) Examples of individuals with unsuccessful eclosion, C) image of an adult with morphological measurements of body and organ size, thorax width (orange arrow with rectangle bounding box), forewing measurement using 11 landmarks (red dots with cyan numbers), proboscis length (red arrow), antennal stalk length (light blue arrow). Inset: 3D rendering of a butterfly head with segmented left eye area (indicated by gold lines), D) example of major wing deformities of the left and the right fore-wings and hind-wings.

grid paper reference and were measured in mm, in FIJI-image J, 64-bit Java 1.8.0\_172 (Schindelin et al., 2012). The reconstructed X-ray scan images containing the left compound eyes were cropped, and the optimal contrast was set in Drishti 2.6.4 image processing software (Limaye, 2012). The eye volumes were then segmented, and the 3D surface area (Fig. 1C) was measured in  $\mu\text{m}^2$  using Amira 6.2.0 (Thermo Fisher Scientific, Waltham, MA, USA). Organs damaged through handling and dissection process were not included in the analysis, leading to variations in the sample sizes for each measurement (Appendix A-Table S3).

#### 2.4. Data analysis

All statistical analyses were conducted using R version 4.0.5 (R Core Team, 2021). Linear mixed models using the R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) were used to construct the models for all morphological traits. In each model, temperature and sex, as well as their interaction, were included as fixed factors. Reduced models were subsequently used if the fixed factors did not significantly improve the model. Non-significant interaction terms were removed from the models. The likelihood ratio test was used to achieve the definitive models using type I ANOVAs in all the analyses. To achieve residual normality of the response variables ‘eye area’ and ‘thorax width’, these variables were transformed to  $\log_{10}$ . Post-hoc pairwise comparisons were used when significant interactions were found using the emmeans package (Lenth et al., 2018). Final significant results were extracted from the models using type II ANOVAs from the car package (Fox and Weisberg, 2018). Mortality rate was analysed using a generalized non-linear model with a binomial error distribution. Pupal development time was analysed using a ranked-based Kruskal-Wallis one-way ANOVA due to variance heterogeneity in the response

variable. To specifically assess the variation of wing shape, we conducted a Procrustes ANOVA (Goodall, 1991) using the procD.lm function from geomorph package (Adams and Otarola-Castillo, 2013). Where reported in the Results, values are given as mean  $\pm$  standard deviation.

### 3. Results

#### 3.1. Mortality rate and pupal development

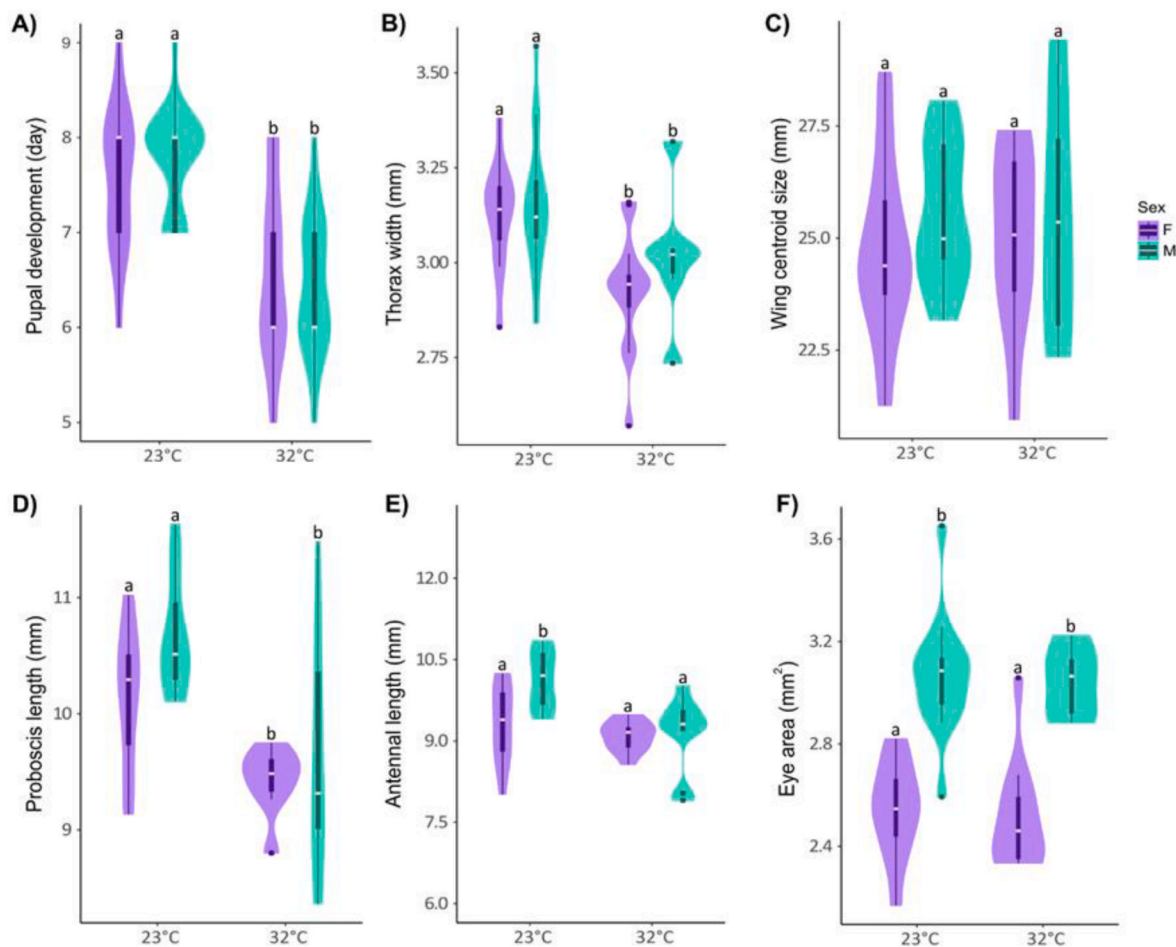
The mortality rate was higher within individuals reared in 32 °C (7/36, 19%) compared to 23 °C (1/36, 3%) during the pupal stage ( $X_1^2 = 5.68$ ,  $P = 0.017^*$ ). Sex did not influence mortality rate within temperature groups ( $X_1^2 = 0.83$ ,  $P = 0.36$ ).

The development time from pupa to eclosed adult was significantly shorter (Fig. 2A,  $\text{kw } X_1^2 = 25.97$ ,  $P < 0.0001^{***}$ ) for pupae reared in 32 °C ( $6.5 \pm 0.80$  days) compared to 23 °C ( $7.7 \pm 0.63$  days).

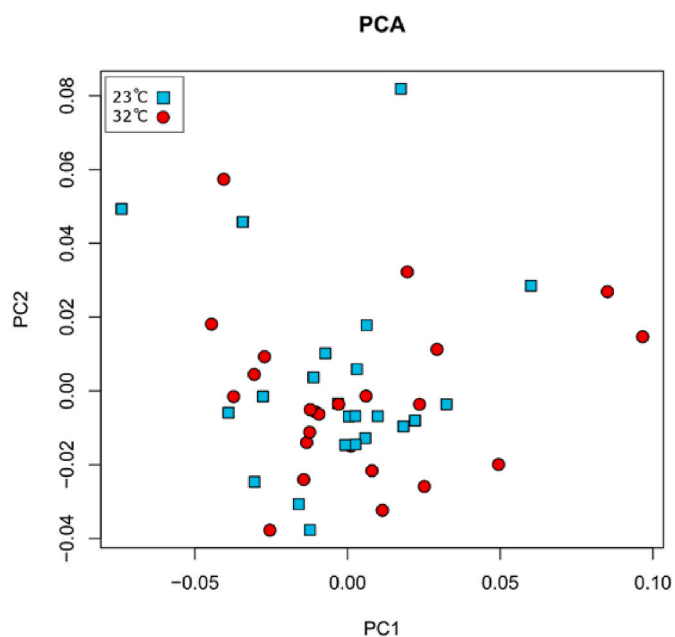
#### 3.2. Body and organ morphology

Thorax width was significantly smaller in the 32 °C ( $2.95 \pm 0.2$  mm) temperature group than 23 °C ( $3.17 \pm 0.2$  mm, Fig. 2B,  $F_{47,1} = 11.97$ ,  $P = 0.001^{**}$ ) but was not affected by sex (Fig. 2B,  $F_{47,1} = 1.08$ ,  $P = 0.304$ ).

Forewing centroid size was not affected by temperature treatment (Fig. 2C,  $F_{47,1} = 0.07$ ,  $P = 0.793$ ) or by sex (Fig. 2C,  $F_{47,1} = 1.19$ ,  $P = 0.281$ ). Similarly, the shape of the forewing did not vary between temperature treatments (Fig. 3,  $F_{47,1} = 0.47$ ,  $P = 0.832$ ), although we find a sex specific influence on wing shape ( $F_{47,1} = 6.52$ ,  $P = 0.001^{**}$ ). For individuals with major wing deformities, we found a significant interaction between temperature treatment and sex ( $F_{56,1} = 9.72$ ,  $P = 0.002^{**}$ ), with some indication of a higher proportion of wing deformities in males that developed at 32 °C compared to 23 °C ( $P = 0.08$ ,



**Fig. 2.** Violin and box plots of the effect of developmental temperature and sex on the size of body parts and sensory organs in *Pieris napi*. A) Pupal development time ( $n = 62$ ), B) thorax width ( $n = 47$ ), C) wing centroid size ( $n = 47$ ), D) proboscis length ( $n = 42$ ), E) antennal length ( $n = 48$ ), F) and eye surface area ( $n = 45$ ), in females (purple) and males (green). The box plots represent the median (bright line) as well as the 25th and 75th percentiles, maximum and minimum values (whiskers) and outliers (circles). Different letters above the violin plots indicate significant differences.



**Fig. 3.** Principle component analysis of the wing landmarks showing the correlations between wing shape in the two temperature treatments in *P. napi*.

post hoc pairwise-comparison analysis). Out of 56 forewing measurements ( $n = 32$ , from 23 °C and  $n = 24$  from 32 °C), major wing deformities were found in 4 individuals from 23 °C, and in 6 individuals from 32 °C.

Proboscis length was significantly affected by temperature treatment (Fig. 2D,  $F_{42,1} = 18.64$ ,  $P = 0.0001^{***}$ ) and sex (Fig. 2D,  $F_{42,1} = 4.371$ ,  $P = 0.043^*$ ), with both males and females from the 32 °C treatment having shorter proboscides ( $9.53 \pm 0.7$  mm) than those from the 23 °C treatment ( $10.44 \pm 0.6$  mm, Fig. 2D).

Antennal length was significantly affected by the interaction between temperature treatment (32 °C:  $9.11 \pm 0.5$  mm, 23 °C:  $9.81 \pm 0.7$  mm) and sex (Table S2,  $F_{48,1} = 4.76$ ,  $P = 0.034^*$ ). Post hoc pairwise-comparisons revealed that male butterflies reared in the higher temperature developed shorter antennae (Fig. 2E,  $P = 0.0008^{***}$ ), while antennal length did not significantly differ between females from the two temperature treatments (Fig. 2E,  $P = 0.758$ ).

Finally, eye surface area was not significantly affected by temperature treatment (Fig. 2F,  $F_{45,1} = 0.19$ ,  $P = 0.663$ ), (32 °C:  $2.7 \pm 0.3$  mm<sup>2</sup>, 23 °C:  $2.84 \pm 0.3$  mm<sup>2</sup>) but a sexual dimorphism in this trait was observed within treatments (Fig. 2F,  $F_{45,1} = 73.09$ ,  $P < 0.0001^{***}$ ) with males having larger eyes than females.

#### 4. Discussion

Exposure to elevated temperature during the last stage of metamorphosis affected the development and survival rate in *Pieris napi*. We

found that, at 32 °C, there was a 6.3 times increase in mortality, indicating that the survival of developing pupae is extremely sensitive to increases in ambient temperature. As this is a temperature that *P. napi* is increasingly being exposed to during summer in their natural habitat (Appendix A, Figure S2), our findings indicate that the mortality rates of this species, and indeed other temperate butterfly species, is likely to increase with global warming.

Individuals that developed at 32 °C also eclosed faster than those at 23 °C. This could be ecologically beneficial as it would increase the chance of finding mates and decrease competition for mates and food, especially for adult males (Fagerström and Wiklund, 1982). However, ectothermic insects who develop faster throughout their life cycle due to higher temperature are often smaller in size (Atkinson, 1994; Kingsolver and Huey, 2008) and have less energy storage for foraging over longer distances (Greenleaf et al., 2007). Indeed, we found that individuals of both sexes reared at 32 °C had smaller thorax widths compared to those reared in 23 °C. Thorax width is an important proxy of body size as it is an indicator of the thoracic muscle mass necessary for flight. Butterflies with the smaller thorax widths are therefore likely to have lower flight endurance. This, in turn, would affect dispersal ability and mating success (Gyulavári et al., 2014; Kehl et al., 2015), as well as manoeuvrability and take-off capability (Berwaerts and Van Dyck, 2004; Almbro and Kullberg, 2008; Stjernholm and Karlsson, 2008), which are factors that are important for predator avoidance (Jantzen and Eisner, 2008).

In contrast to thorax width, exposure to 32 °C did not appear to affect the development of the wings as it had no effect on forewing size and shape. This indicates that, despite faster developmental times and smaller thorax widths, individuals that developed at 32 °C grew wings that were relatively larger with respect to their body size than those that developed in 23 °C. The discrepancy between the size of the wing and thorax can be an indication that these organs have different thermal sensitivities during pupal development (French et al., 1998), with wing growth being less sensitive to temperature variations. This finding is not consistent with previous work on other butterfly species or indeed in bumblebees, since exposure to high temperature in early and pupal stages of development commonly results in smaller wings (Bowden et al., 2015; Gérard et al., 2018; Soule et al., 2020). The maintenance of wing size in high temperature observed in our study suggests that wing development in *P. napi* has a high thermal tolerance. Since it was not possible to analyse the shape of highly deformed wings, our results are only applicable to the wings of individuals that were less affected by the high developmental temperature (i.e. those that eclosed with little to no wing deformity). When the rate of wing deformity was compared between the two temperature groups, we found an interaction effect of temperature and sex, meaning that wing deformity was not clearly a result of higher temperature in both sexes. Desiccation is one possible cause of the wing deformity we observed, as it often co-occurs with rising temperature and during wing expansion in butterflies (Scriber et al., 2002; Mengesha et al., 2011), although further investigations with a larger sample size would be necessary to explore how temperature and desiccation affect wing deformity during last stages of development in butterflies. Surprisingly, studies on the effect of developmental temperature on wing deformity and wing desiccation in butterflies are limited, despite the impact these factors may have on flight performance and flight dynamics.

Exposure to elevated temperature during development also caused a reduction in proboscis length in both sexes and in antennal length in males. This could be a direct consequence of a reduction in body or head capsule size (Stevens, 2004), higher temperature sensitivity of these sensory organs during development, or the cost of energy trade-off within tissues in reaction to high temperature. Proboscis length plays an important role in plant-pollinator interactions as it is tightly linked to

the floral morphology of visited flowers (Gérard et al., 2020), such as corolla depth (Harder, 1988; Krenn et al., 2001; Szigeti et al., 2020). Here, the differences in temperature treatment caused approximately 1 mm difference in proboscis length. While it is not clear exactly how much such a difference in length would affect feeding behaviour, it is possible that such changes in proboscis length disrupt important plant-pollinator relationships due to the mismatch between flower corolla tube and proboscis morphology (Gérard et al., 2020). For example, in Apollo butterflies, individuals with shorter proboscides lost their ability to feed from flowers with deep corollas and, as a result, had to use alternative floral resources, likely affecting pollination of those flowers and the time taken to forage (Szigeti et al., 2020). In addition, as the shape and size of the sensory organs often affect their function, and their sensitivity in particular, temperature-induced proboscis reduction may decrease gustatory sensitivity to nectar in some flower species.

Reduction in antennal length due to exposure to elevated temperature in males may reduce the olfactory sensilla numbers, which would impair their olfactory sensitivity. For example, a decrease in olfactory sensitivity with body size (and presumably antennal length) has been found in bumblebee workers (Spaethe et al., 2007). In males, increases in temperature led to a decrease in antennal length while in females this relationship was not as distinct. One possible explanation is that females prioritise antennal growth as it is important for finding plants for oviposition and larval feeding, although further work is necessary to understand what underlies sex-specific differences in the temperature sensitivity of antennal growth.

Surprisingly, we did not find any effect of elevated temperature on the eye size in *P. napi*. Our findings suggest that, similar to wings, the processes that affect development in *P. napi* eyes are not thermally sensitive. Investing in an adequate eye size is likely essential for maintaining the sensitivity and resolution necessary to locate mates, flowers and host plants across the range of light intensities that butterflies would normally experience in their habitat. To our knowledge the thermal sensitivity and thermal plasticity of insect eyes has not previously been investigated but it would be interesting to explore if the thermal sensitivity of eye development observed here is common among other species and taxa.

While we did not explicitly explore the mechanisms underlying the change in organ size in response to temperature observed in this study, some potential explanations have been proposed by earlier studies. Firstly, temperature could affect cell size and cell division during development, leading to variation in the size of the organs. For instance, Azevedo et al. (2002) and French et al. (1998) found that, in *Drosophila melanogaster*, changes in temperature during development affected the size of the thorax, wings, legs and eyes by mostly affecting the epidermal cell size but not the cell numbers. Secondly, increasing temperature can increase the rate of juvenile hormone clearance via enzymes during the pupal stage (Radmacher and Strohm, 2010), which would result in a smaller body size (and possibly smaller organs).

One additional factor that might affect the changes in the size of the body and organs observed here is water loss due to the lower humidity in the 32 °C treatment (30% in the 23 °C and 20% in the 32 °C). The lower relative humidity in 32 °C would result in a higher vapor pressure deficit (VPD) of 3808 Pa compared to a VPD of 1970 Pa in 23 °C. This difference in VPD might have increased the water evaporation rate from the tissue of the developing pupae in the higher temperature treatment and thus caused the decrease we found in thorax size and some of the sensory organs due to desiccation. The body water content and cuticular permeability (for gas exchange) in arthropods can be affected by multiple parameters such as ambient temperature and humidity, developmental life stage, physiological state, as well as species and sex (Mack and Appel, 1986; Hadley, 1994; Mazer and Appel, 2001; Edney, 2012;

Williams et al., 2012). Cuticular permeability in the pupal stage has been shown to be lower compared to other stages of the insect life cycle (Edney, 2012). For example, in Lesser Cornstalk Borers, *Elasmopalpus lignosellus*, cuticular permeability was lowest during the pupal stage, while the total body water content and water permeability was highest during larval stage (Mack and Appel, 1986). Our finding that many individuals in the 32 °C treatment eclosed with normal organ morphology and unaffected eye and wing size (Appendix A, Figure S3) might therefore indicate that these pupae were able to retain sufficient water for proper development under this condition. It is nonetheless important to note that temperature alone might not explain the reduction in body and some of the sensory organ size in this study and the effect of differences in humidity should also be taken into consideration when interpreting these results. While the effect of developmental temperature on insects as a result of rising global temperatures has received more attention recently, the role of humidity and its interaction with elevated temperature is less clear and needs further investigation.

## 5. Conclusion

In this study, we showed that exposure to elevated temperatures that arise as a result of global warming has negative consequences on the survival and development time in a temperate butterfly, *Pieris napi*. While widespread temperate species are considered to be less affected by global warming than tropical butterflies (Klockmann et al., 2016, 2017), our results provide some evidence that temperate species will indeed be vulnerable to the increasing temperatures that are becoming increasingly common in their habitats. Exposure to elevated temperature accompanied with reduced humidity during the last stage of development also affected on the morphology of body and sensory organs in *P. napi*. However, these effects were not the same for all organs and exhibited some sex dependence. It is not entirely clear what mechanisms underlie this disparity between organs and sex-specific variations in response to elevated temperatures but perhaps differences in the thermal sensitivity of the tissues from which they arise during metamorphosis is one of the main factors. It is important to note that sensory systems are multimodal and that information from different organs is combined in order to drive appropriate behaviours. For example, a combination of visual, olfactory and gustatory cues stimulates a feeding response in butterflies (Andersson, 2003; Omura and Honda, 2005; Sourakov et al., 2012). Accordingly, a reduction in size (and any potential loss in sensitivity) of one or several organs could influence the function of the whole system or disrupt the transmission of the signal/s to the central nervous system. Behavioural assays would be beneficial to further understand the effect of high developmental temperature on the function and sensitivity of sensory organs due to morphological changes and their impact on population viability of insect pollinators.

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## Authors' contributions

**Zahra Moradinour:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization, Project administration **Emily Baird:** Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition **Christer Wiklund:** Validation, Investigation, Resources, Writing - Review & Editing **Maxence Gérard:** Validation, Formal analysis, Writing - Review & Editing **Arttu Miettinen:** Investigation, Resources, Writing - Review & Editing.

## Declaration of competing interest

The authors declare to have no conflict of interest.

## Data availability

The data provided for this paper is available in Mendeley digital common data: DOI: 10.17632/j7jrzt34bn.6.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2023.103721>.

## References

- Adams, D.C., Otarola-Castillo, E., 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* 4, 393–399. <https://doi.org/10.1111/2041-21X.12035>.
- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Ital. J. Zool.* 71, 5–16. <https://doi.org/10.1080/11250000409356545>.
- Agrawal, A.A., Inamine, H., 2018. Mechanisms behind the monarch's decline. *Science* 360, 1294–1296. <https://doi.org/10.1126/science.aat5066>.
- Almbro, M., Kullberg, C., 2008. Impaired escape flight ability in butterflies due to low flight muscle ratio prior to hibernation. *J. Exp. Biol.* 211, 24–28. <https://doi.org/10.1242/jeb.008219>.
- Andersson, S., 2003. Foraging responses in the butterflies *Inachis io*, *Aglais urticae* (Nymphalidae), and *Gonepteryx rhamni* (Pieridae) to floral scents. *Chemoecology* 13, 1–11. <https://doi.org/10.1007/s000409300000>.
- Atkinson, D., 1994. Temperature and organism size: a biological law for ectotherms? *Adv. Ecol. Res.* 25, 1–58. [https://doi.org/10.1016/S0065-2504\(08\)60212-3](https://doi.org/10.1016/S0065-2504(08)60212-3).
- Azevedo, R.B.R., French, V., Partridge, L., 2002. Temperature modulates epidermal cell size in *Drosophila melanogaster*. *J. Insect Physiol.* 48 (2), 231–237. [https://doi.org/10.1016/S0022-1910\(01\)00168-8](https://doi.org/10.1016/S0022-1910(01)00168-8).
- Bai, Y., Ma, L.B., Xu, S.-Q., Wang, G.-H., 2015. A geometric morphometric study of the wing shapes of *Pieris rapae* (Lepidoptera: Pieridae) from the Qinling Mountains and adjacent regions: an environmental and distance-based consideration. *Fla. Entomol.* 1, 162–169. <https://doi.org/10.1653/024.098.0128>.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Berwaerts, K., Van Dyck, H., 2004. Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria*. *Oecologia* 141, 536–545. <https://doi.org/10.1007/s00442-004-1661-9>.
- Bowden, J.J., Eskildsen, A., Hansen, R.R., Olsen, K., Kurlle, C.M., Høye, T.T., 2015. High-Arctic butterflies become smaller with rising temperatures. *Biol. Lett.* 11, 20150574. <https://doi.org/10.1098/rsbl.2015.0574>.
- Chinery, M., 1998. *Collins Guide to Butterflies: A Photographic Guide to the Butterflies of Britain and Europe*. Harper Collins Publishers, New York, USA.
- Corbet, S.A., 2000. Butterfly nectaring flowers: butterfly morphology and flower form. *Entomol. Exp. Appl.* 96, 289–298. <https://doi.org/10.1046/j.1570-7458.2000.00708.x>.
- Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L.A., Gemmill-Herren, B., Howlett, B.G., Imperatriz-Fonseca, V.L., Johnson, S.D., Kovács-Hostyánszki, A., Kwon, Y.J., Lattorff, H.M.G., Lungharwo, T., Seymour, C.L., Vanbergen, A.J., Potts, S.G., 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nat Ecol Evol* 5, 1453–1461. <https://doi.org/10.1038/s41559-021-01534-9>.
- Edney, E.B., 2012. *Water Balance in Land Arthropods*, vol. 9. Springer Science & Business Media.
- Fagerström, T., Wiklund, C., 1982. Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia* 52 (2), 164–166. <https://doi.org/10.1007/BF00363830>.
- Fox, J., Weisberg, S., 2018. *An R Companion to Applied Regression*, 3d edition. Sage publications.
- French, V., Feast, M., Partridge, L., 1998. Body size and cell size in *Drosophila*: the developmental response to temperature. *J. Insect Physiol.* 44, 1081–1089. [https://doi.org/10.1016/S0022-1910\(98\)00061-4](https://doi.org/10.1016/S0022-1910(98)00061-4).
- Gardner, K.E., Foster, R.L., O'Donnell, S., 2007. Experimental analysis of worker division of labor in bumblebee nest thermoregulation (*Bombus huntii*, Hymenoptera: apidae). *Behav. Ecol. Sociobiol.* 61, 783–792. <https://doi.org/10.1007/s00265-006-0309-7>.
- Gérard, M., Michez, D., Debat, V., Fullgrave, L., Meeus, I., Piot, N., Sculfort, O., Vastrade, M., Smaghe, G., Vanderplanck, M., 2018. Stressful conditions reveal decrease in size, modification of shape but relatively stable asymmetry in bumblebee wings. *Sci. Rep.* 8, 1–10. <https://doi.org/10.1038/s41598-018-33429-4>.
- Gérard, M., Vanderplanck, M., Wood, T., Michez, D., 2020. Global warming and plant–pollinator mismatches. *Emerg Top Life Sci* 4, 77–86. <https://doi.org/10.1042/ETLS20190139>.
- Gérard, M., Guiraud, M., Cariou, B., Henrion, M., Baird, E., 2023. Elevated developmental temperatures impact the size and allometry of morphological traits of

- the bumblebee *Bombus terrestris*. *J. Exp. Biol.* 226 (8), jeb245728. <https://doi.org/10.1242/jeb.245728>.
- Goodall, C.R., 1991. Procrustes methods in the statistical analysis of shape. *J. Roy. Stat. Soc.* 53, 285–339. [10.1111/j.2517-6161.1991.tb01825.x](https://doi.org/10.1111/j.2517-6161.1991.tb01825.x).
- Goulson, D., Cory, J.S., 1993. Flower constancy and learning in foraging preferences of the green-veined white butterfly *Pieris napi*. *Ecol. Entomol.* 18 (4), 315–320. <https://doi.org/10.1111/j.1365-2311.1993.tb01107.x>.
- Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153, 589–596. <https://doi.org/10.1007/s00442-007-0752-9>.
- Gyulavári, H.A., Therry, L., Dévai, G., Stoks, R., 2014. Sexual selection on flight endurance, flight-related morphology and physiology in a scrambling damselfly. *Evol. Ecol.* 28, 639–654. <https://doi.org/10.1007/s10682-014-9703-1>.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. CUP Archive.
- Harder, L.D., 1988. Choice of individual flowers by bumble bees: interaction of morphology, time and energy. *Behaviour* 104 (1–2), 60–76. <https://doi.org/10.1163/156853988X00601>.
- Huang, Y., McPherson, J., Jiggins, C.D., Montejó-Kovacevich, G., 2022. Effects of Temperature on the Development of *Heliconius erato* Butterflies. *bioRxiv*, p. 2022. <https://doi.org/10.1101/2022.12.07.519472>, 12.
- Jantzen, B., Eisner, T., 2008. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. *Proc. Natl. Acad. Sci. USA* 105, 16636–16640. <https://doi.org/10.1073/pnas.0807223105>.
- Kehl, T., Bensch, J., Böhm, F., Kniepkamp, B.O., Leonhardt, V., Schwieger, S., Fischer, K., 2015. Fat and sassy: factors underlying male mating success in a butterfly. *Entomol. Exp. Appl.* 155, 257–265. <https://doi.org/10.1111/eea.12305>.
- Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., Gall, L.F., Sikes, D.S., Pantoja, A., 2015. Climate change impacts on bumblebees converge across continents. *Science* 349, 177–180. <https://doi.org/10.1126/science.aaa7031>.
- Kingsolver, J., Huey, R., 2008. Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* 10 (2), 251–268, 10: 251–268.
- Klockmann, M., Schröder, U., Karajoli, F., Fischer, K., 2016. Simulating effects of climate change under direct and diapause development in a butterfly. *Entomol. Exp. Appl.* 158, 60–68. <https://doi.org/10.1111/eea.12380>.
- Klockmann, M., Karajoli, F., Kuczyk, J., Reimer, S., Fischer, K., 2017. Fitness implications of simulated climate change in three species of copper butterflies (Lepidoptera: lycaenidae). *Biol. J. Linn. Soc.* 120, 125–143. <https://doi.org/10.1111/bj.12846>.
- Krenn, H.W., Zulka, K.P., Gatschnegg, T., 2001. Proboscis morphology and food preferences in nymphalid butterflies (Lepidoptera: nymphalidae). *J. Zool.* 254, 17–26. <https://doi.org/10.1017/S0952836901000528>.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Software* 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Larsdotter-Mellström, H., Wiklund, C., 2015. Different mating expenditure in response to sperm competition risk between generations in the bivoltine butterfly *Pieris napi*. *Behav. Ecol. Sociobiol.* 69, 1067–1074. <https://doi.org/10.1007/s00265-015-1919-8>.
- Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. *Emmeans: Estimated Marginal Means, Aka Least-Squares Means*. R package version 1.
- Limaye, A., 2012. *Drishhti: a volume exploration and presentation tool*, 85060X Dev X-ray Tomogr VIII, 8506, 10.1177/12.935640.
- Mack, T.P., Appel, A.G., 1986. Water relations of immature and adult lesser cornstalk borers, *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 79 (4), 579–582. <https://doi.org/10.1093/aesa/79.4.579>.
- Mazer, C.L., Appel, A.G., 2001. Water loss and desiccation tolerances of longwing butterflies (Lepidoptera: nymphalidae). *Environ. Entomol.* 30 (4), 631–636. <https://doi.org/10.1603/0046-225X-30.4.631>.
- McDonald, J., Ghosh, S.M., Gascoigne, S.J., Shingleton, A.W., 2018. Plasticity through canalization: the contrasting effect of temperature on trait size and growth in *Drosophila*. *Front. Cell Dev. Biol.* 6, 156. <https://doi.org/10.3389/fcell.2018.00156>.
- Mengesha, T.E., Vallance, R.R., Mittal, R., 2011. Stiffness of desiccating insect wings. *Bioinspiration Biomimetics* 6, 014001. <https://doi.org/10.1088/1748-3182/6/1/014001>.
- Molet, M., Péronnet, R., Couette, S., Canovas, C., Doums, C., 2017. Effect of temperature and social environment on worker size in the ant *Temnothorax nylanderi*. *J. Therm. Biol.* 67, 22–29. <https://doi.org/10.1016/j.jtherbio.2017.04.013>.
- Omura, H., Honda, K., 2005. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* 142, 588–596. <https://doi.org/10.1007/s00442-004-1761-6>.
- Perl, C.D., Johansen, Z.B., Moradinour, Z., Guiraud, M., Restrepo, C.E., Jie, V.W., Miettinen, A., Baird, E., 2022. Heatwave-like events during development are sufficient to impair bumblebee worker responses to sensory stimuli. *Front. Ecol. Evol.* 9, 776830. <https://doi.org/10.3389/fevo.2021.776830>.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Radmacher, S., Strohm, E., 2010. Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie* 41 (2), 169–177. <https://doi.org/10.1051/apido/2009064>.
- Rohlf, F.J., 2015. The tps series of software. *Hystrix* 26, 9–12, 4404/hystrix-26.1-11264.
- Rohlf, F.J., Loy, A., Corti, M., 1996. Morphometric analysis of old world talpidae (Mammalia, Insectivora) using partial-warp scores. *Syst. Biol.* 45 (3), 344–362. <https://doi.org/10.1093/sysbio/45.3.344>.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. <https://doi.org/10.1038/nmeth.2019>.
- Scriber, J.M., Keefover, K., Nelson, S., 2002. Hot summer temperatures may stop movement of *Papilio canadensis* butterflies and genetic introgression south of the hybrid zone in the North American Great Lakes region. *Ecography* 25, 184–192. <https://doi.org/10.1034/j.1600-0587.2002.250206.x>.
- Shingleton, A.W., Estep, C.M., Driscoll, M.V., Dworkin, I., 2009. Many ways to be small: different environmental regulators of size generate distinct scaling relationships in *Drosophila melanogaster*. *Proc. Royal Soc. B* 276, 2625–2633. <https://doi.org/10.1098/rspb.2008.1796>.
- Soroye, P., Newbold, T., Kerr, J., 2020. Climate change contributes to widespread declines among bumble bees across continents. *Science* 367, 685–688. <https://doi.org/10.1126/science.aax8591>.
- Soule, A.J., Decker, L.E., Hunter, M.D., 2020. Effects of diet and temperature on monarch butterfly wing morphology and flight ability. *J. Insect Conserv.* 24, 961–975. <https://doi.org/10.1007/s10841-020-00267-7>.
- Sourakov, A., Duehl, A., Sourakov, A., 2012. Foraging behavior of the blue morpho and other tropical butterflies: the chemical and electrophysiological basis of olfactory preferences and the role of color. *Psyche* 2012, 378050, 10.1155/2012/378050.
- Spaeth, J., Brockmann, A., Halbig, C., Tautz, J., 2007. Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften* 94 (9), 733–739. <https://doi.org/10.1007/s00114-007-0251-1>.
- Steigenga, M.J., Fischer, K., 2009. Fitness consequences of variation in developmental temperature in a butterfly. *J. Therm. Biol.* 34, 244–249. <https://doi.org/10.1016/j.jtherbio.2009.03.001>.
- Stevens, D.J., 2004. Pupal development temperature alters adult phenotype in the speckled wood butterfly, *Pararge aegeria*. *J. Therm. Biol.* 29, 205–210. <https://doi.org/10.1016/j.jtherbio.2004.02.005>.
- Stjernholm, F., Karlsson, B., 2008. Flight muscle breakdown in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). *Eur. J. Entomol.* 105, 87–91. <https://doi.org/10.14411/eje.2008.012>.
- Stuhldreher, G., Hermann, G., Fartmann, T., 2014. Cold-adapted species in a warming world—an explorative study on the impact of high winter temperatures on a continental butterfly. *Entomol. Exp. Appl.* 151 (3), 270–279. <https://doi.org/10.1111/eea.12193>.
- Szigeti, V., Vajna, F., Kőrösi, Á., Kis, J., 2020. Are all butterflies equal? Population-wise proboscis length variation predicts flower choice in a butterfly. *Anim. Behav.* 163, 135–143. <https://doi.org/10.1016/j.anbehav.2020.03.008>.
- Tamir, A., Getu, E., Jembere, B., Bruce, T., 2012. Effect of temperature and relative humidity on the development and fecundity of *Chilo partellus* (Swinhoe) (Lepidoptera: crambidae). *Bull. Entomol. Res.* 102 (1), 9–15. <https://doi.org/10.1017/S0007485311000307>.
- Taylor, G.J., Tichit, P., Schmidt, M.D., Bodey, A.J., Rau, C., Baird, E., 2019. Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity. *Elife* 8, e40613. <https://doi.org/10.7554/eLife.40613>.
- The Swedish Agency for Marine and Water Management and SMHI. <https://www.smhi.se/q/Stockholm/2673730>.
- von Schmalensee, L., Caillault, P., Gunnarsdóttir, K.H., Gotthard, K., Lehmann, P., 2023. Seasonal specialization drives divergent population dynamics in two closely related butterflies. *Nat. Commun.* 14 (1), 3663. <https://doi.org/10.1038/s41467-023-39359-8>.
- Williams, C.M., Hellmann, J., Sinclair, B.J., 2012. Lepidopteran species differ in susceptibility to winter warming. *Clim. Res.* 53 (2), 119–130. <https://doi.org/10.3354/cr01100>.
- Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. *One Earth* 4, 114–123. <https://doi.org/10.2307/3565338>.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, W., 2012. *Geometric Morphometrics for Biologists: a Primer*. Elsevier Academic press, San Diego.