

Developmental temperature predicts body size, flight, and pollen load in a widespread butterfly

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Abstract

1. Most of our understanding of the effects of climate warming on insect body size comes from laboratory experiments. Whether these studies predict patterns in nature is largely unknown.
2. Here we examine the relevance of laboratory warming experiments for wild populations of the butterfly *Pieris rapae*. We tested two predictions: (i) butterflies reared at warmer temperatures in the laboratory should attain smaller adult sizes and have reduced flight ability, and (ii) in nature, this trait combination should lead to smaller butterflies visiting fewer flowers and accumulating less pollen.
3. Overall, we found that warm-reared butterflies were indeed smaller and flew more slowly compared to colder-reared conspecifics. Additionally, wild-caught small butterflies carried fewer, and a lower diversity of pollen grains compared to larger butterflies.
4. Our warming experiments thus largely predicted pollen collection patterns in wild *P. rapae*.
5. This study demonstrates that increased temperatures will likely have important consequences for butterfly-plant interactions in nature.

KEYWORDS

body size, experiment, flight, *Pieris rapae*, pollen, warming

INTRODUCTION

Insects are involved in all known ecological processes and thus their responses to ongoing global warming will likely have wide-reaching implications (Wilson & Fox, 2021). Given their large population sizes, relatively short generation times (for many taxa), and highly plastic life histories, insects are expected to respond rapidly, both ecologically and evolutionarily, to increased temperatures. Several studies have demonstrated that in response to climate warming, insect distributions have expanded poleward (Hickling et al., 2005; Li et al., 2014; Parmesan et al., 1999) and up-slope (Chen et al., 2009; Keret et al., 2020; Kerr et al., 2015; Menéndez et al., 2014; Wilson et al., 2005). Springtime emergence of insects has also advanced (Gordo & Jose Sanz, 2009; Hällfors et al., 2021; Hassall et al., 2007;

Visser et al., 2006) and body sizes have decreased (Bowden et al., 2015; Nooten & Rehan, 2020; Tseng et al., 2018).

The effect of warming temperatures on insect body size is of particular concern (Gardner et al., 2011) because this trait is tightly correlated with ecological function in nature. For example, larger dung beetles transfer more nutrients into soil (Stanbrook et al., 2021), larger-bodied pollinators carry more pollen (Földesi et al., 2021) and transmit more parasites (Van Wyk et al., 2021), and larger mosquitoes can ingest a greater volume of blood (Rocha-Santos et al., 2021). Additionally, larger insects often have higher fecundity (Honěk, 1993), fly longer distances (Jahant-Miller et al., 2022; Yang et al., 2016), and have higher mating success (Zhang et al., 2021) than their smaller conspecifics.

Despite the knowledge that warming-mediated shifts in body size are likely to have significant consequences for many ecological

processes, there are few studies that directly link the changes in body size caused by warming to ecological function in natural settings. The existing studies that fall under this broad research umbrella can typically be grouped into those that: (a) use laboratory experiments to examine the effect of temperature on body size, fecundity, and sometimes flight (Costanzo et al., 2018; Jardeleza et al., 2022; Ohba et al., 2020; Schneider et al., 2020; Soule et al., 2020; Terada et al., 2019; Wonglersak et al., 2021; Xi et al., 2016), versus (b) those that examine correlations between insect body size and ecological functions such as pollination, dispersal, and nutrient cycling (Földesi et al., 2021; Stanbrook et al., 2021; Yang et al., 2016). Here, our goal is to join these two lines of research. We first use laboratory experiments to examine the effects of warming on body size and flight in the widespread butterfly species *Pieris rapae* (Lepidoptera, Pieridae). We focus on these traits because of their clear links to fecundity, mating success, resource acquisition, dispersal, and plant-insect interactions (Almbro & Kullberg, 2012; Bauerfeind & Fischer, 2008; Duploux et al., 2018; Larranaga et al., 2019; Niitepöld, 2019). Second, we use the results from the experiment to predict how butterfly wing size should affect pollen accumulation and diversity in *P. rapae*, and we test these predictions using a correlational study on wild-caught *P. rapae*. Our study is unique in several ways: (a) it tests the effects of warming on insect flight, (b) it tests the relevance of controlled laboratory warming experiments for wild populations, and (c) it examines the implications of temperature-mediated decreases in insect body size for plant-insect interactions.

Several laboratory warming experiments have demonstrated that insects reared at warmer temperatures attain a smaller adult size (Honěk, 1993). In Lepidoptera specifically, the effects of warming have been mixed. For example, *Papilio xuthus*, *Lycaena tityru*, *Bicyclus anynana*, *Pieris napi*, and *Parage aergia* decreased in size with warming (Bauerfeind & Fischer, 2013; Fischer et al., 2014; Klockmann et al., 2016; Komata & Sota, 2017; Sibly et al., 1997), while *Dryas iulia* and *Bicyclus anynana* increased (Karl et al., 2011; Mega, 2014), and *Parage aergeria* showed no change (Stevens, 2004). With respect to *Pieris rapae* in particular, in some populations body size decreased with warming (Jones et al., 1982; Whitney-Johnson et al., 2005) whereas others, such as those from Japan or warmer locations in the United States, showed little to no response to temperature (Kingsolver et al., 2007; Seiter & Kingsolver, 2013). Plant food type also affected *P. rapae* responses to warming in populations from Taiwan; warming increased *P. rapae* body size when larvae were fed *Rorippa indica*, but there was no effect of warming when larvae were fed *Brassica oleracea* (Lin et al., 2018). Given that we used individuals from a wild population from Vancouver, British Columbia, which is classified as temperate coastal rainforest, we expected *P. rapae* to decrease in size, and have smaller wings when grown at warmer temperatures.

The relationship between body size and flight has not been quantified in *Pieris rapae* but monarchs (*Danaus plexippus*) from long-distance migratory populations have longer wings than those that do not migrate (Altizer & Davis, 2010; Yang et al., 2016), and wing size was positively correlated with flight distance in the invasive *Lymantria dispar* (Jahant-Miller et al., 2022). Given these data, we expected

smaller-winged *P. rapae* to fly slower and for shorter distances than their larger-winged counterparts. With respect to how body or wing size might affect pollen collection in wild *P. rapae*, previous studies on bees and flies have found positive relationships between body size and pollen abundance (Cullen et al., 2021). We were unable to find any existing data on the relationship between body size, flight, and pollen accumulation in butterflies. However, some hawkmoths show positive correlations between proboscis length and pollen load, and body size is positively correlated with proboscis length in many butterflies, suggesting that larger individuals may carry more pollen (Corbet, 2000; Kunte, 2007; Smith et al., 2022). Given the possible positive relationships between body size and pollen load, and between wing size and flight ability, we predicted that smaller-winged/bodied *P. rapae* would visit fewer flowers or flower types and carry fewer pollen grains than larger-winged/bodied conspecifics.

MATERIALS AND METHODS

Study organism

The cabbage white butterfly, *Pieris rapae* (L.) (Pieridae) is a medium-sized butterfly (wingspan 32–47 mm) found across all continents except South America and Antarctica. Their life cycle includes five larval instars and one pupal stage. The species is native to the eastern Mediterranean, and its current distribution and success as an invasive species is linked to the widespread trade and diversification of its brassicaceous larval host plants (Ryan et al., 2019). Although *P. rapae* are considered to be a global pest, they are also pollinators and both males and females visit a variety of plants for nectar (Lazri & Barrows, 1984; Lewis, 1989).

Development time from egg to adult depends on temperature and ranges from 25–40 days. Pupal and adult mass have been shown to increase or decrease with warming, and this variation in sensitivity to temperature appears to be region-specific (Kingsolver et al., 2007; Seiter & Kingsolver, 2013). *Pieris rapae* can fly 0.7–2 km per day (Chew, 1981; Jones et al., 1980) and European populations of *P. rapae* are migratory (Richards, 1940).

Pieris rapae rearing and temperature treatments

We conducted this experiment twice using identical methods. Experiment 1 began in August 2020 and started with approximately 30 first instars per temperature treatment. Experiment 2 began in September 2020 and started with approximately 40 first instars per treatment. *Pieris rapae* body size is known to vary throughout the summer (Kimura & Tsubaki, 1986) and thus by conducting the experiment with two different cohorts of wild-laid eggs, we expected to capture a larger scope of the size variation in these butterflies.

To collect wild-laid eggs, we placed eight pots of organic Lacinato variety kale (*Brassica oleracea*) in the front yard of one of the authors (MT) in Vancouver, British Columbia. Kale plants were 10 cm tall and pots were filled with organic potting soil. Plants, pots, and soil were

purchased from a local garden centre. Wild *P. rapae* were observed laying eggs on these plants daily (MT personal observation).

Eggs were gently removed from the outdoor kale plants using forceps and placed individually on a 5 cm² piece of kale in 4 oz plastic cups at room temperature (21°C–23°C) until they hatched. After hatching, first instar larvae were transferred to temperature-controlled incubators (Panasonic MIR-254) set to 18°C, 24°C, or 30°C, 16:8 h light: dark photoperiod, and low humidity. Previous studies have shown that these temperatures produce clear differences in *P. rapae* body size (Jones et al., 1982). Additionally, Environment Canada Historical Climate Data for the Vancouver International Airport weather station (ID 1108395, climate.weather.gc.ca), lists the average summer (June to August) temperature for 2021 as 18.7°C, the average maximum temperature as 23.1°C, and the highest temperature recorded in each of the three months as 32.4°C, 28.1°C, and 28.9°C. The three temperatures used in this experiment are thus fairly close representations of the average, maximum, and extreme temperatures experienced by this population in this region.

After 9:00 PM, all incubators were programmed to decrease in temperature by 4°C to create a reasonably accurate simulation of a nighttime cool down for this location. Every two hours, we reduced the incubator temperature by 1°C. Temperature was brought back to daytime levels at 5:00 AM. Light was provided by factory-installed fluorescent lights and augmented with one additional 24 W full-spectrum LED bar per incubator (Sunblaster LED, Langley, Canada). The temperatures of the incubators were haphazardly rotated every two weeks to minimise incubator-specific effects. Caterpillar rearing containers were haphazardly moved within incubators daily to minimise within-incubator variation in environmental effects.

Pieris rapae larvae were examined daily. Each day, frass was removed, old kale replaced with fresh kale, and instar number and sex were recorded. Male caterpillars have kidney-shaped yellow spots on the dorsal surface (Underwood, 1994). One day after pupation, pupae were carefully detached from their silk pad and weighed (VWR-214B2, ±0.1 mg). Pupae were returned to the same cup and temperature treatment until adult emergence. After emergence, adult butterflies were kept in 4 oz plastic cups and fed using paper wicks soaked in 20% honey-water solution.

The survival percentage from first instar to adult was approximately 70%. This survival rate is approximately twice as high as that documented in the field (Smith et al., 1987). We were unable to assess flight in 25%–30% of the adults because of malformed wings. Newly emerged adult butterflies need to hang upside down to properly dry and expand their wings. We believe that removing the pupa from their silk tethers in order to weigh them negatively affected their ability to hang upside down to dry their wings. Overall, across the two experiments the total sample size was 101 individuals.

Flight assays

Butterfly flight was assayed using a custom-built flight mill (Figure 1a). Flight mills are commonly used to assess insect flight and have been

used on insect taxa including aphids, bees, moths, butterflies, and mosquitoes (Naranjo, 2019). Our flight mill was modelled after one used for monarch butterflies (Schroeder et al., 2020) and consisted of a lightweight carbon fibre rod (35 cm length, 1.2 mm diameter) attached to a ball bearing (22 mm outer, 8 mm inner diameter). Two extra arms (35 cm and 20 cm) with moveable weights were also attached to the ball bearing for balance. A flag was attached to one of the flight mill arms. During butterfly flight, the flag passed through an infrared beam emitted by a photogate. The photogate was connected to an Arduino Uno microcontroller board, which was attached to a computer. We programmed the Arduino IDE (version 1.8.13) to record time elapsed between each rotation. The flight path was 2.19 m per revolution.

Butterflies were connected to the flight mill via a lightweight tether made from 32-gauge jewellery wire (length: 2.9–4.4 cm, length mean: 3.6 cm; weight: 6.6–11.3 mg; mean weight: 8.7 mg). To attach the tether to individual *P. rapae*, one day after emergence, butterflies were chilled at 5°C for 15 min. After chilling, the tether was glued to the mesothorax using non-toxic hot glue. As the glue dried, we placed a microscope slide on the butterfly's wings and placed a small piece of paper on top of the slides to immobilise the wings and abdomen. Butterflies were returned to their individual 4 oz containers after attachment of the tether. Prior to the flight assay, we used the same non-toxic hot glue to attach the free end of the tether to one flight mill flight arm.

Flights were conducted in the laboratory under artificial light and at room temperature (approximately 22°C). We recorded flight data until butterflies stopped flying for more than 30 seconds. Wire tethers were gently removed after the flight assay and butterflies were frozen at –18°C. For each butterfly, we recorded total distance flown (m) and average flight velocity per revolution (m/s).

Life history and morphological traits of lab-reared *P. rapae*

For both experiments, we recorded total development time (first instar to adult [days]), and pupa mass (mg). We were only able to record adult mass in Experiment 2 due to logistical constraints. In this experiment, wet mass was recorded post chilling and prior to attaching the flight tether. For both experiments, we removed all four wings after the flight assays and photographed them using a Nikon D90 DSLR camera fitted with a 100 mm macro lens. We used ImageJ (Rasband, 2016) to measure forewing and hindwing length and width, and total wing area (Figure 1b, [Flockhart et al., 2017]). We measured wing area by using the 'freehand' tool to draw an outline of the left and right forewings and hindwings, and then used the 'Measure' tool to calculate area. We recorded wing loading (mass/wing size) for individuals from Experiment 2.

Wing size and pollen counts from wild-caught *P. rapae*

To test how well the results from the laboratory warming experiments predicted pollen collection behaviour in wild *P. rapae*, in summer 2021, we caught 100 butterflies from the University of British

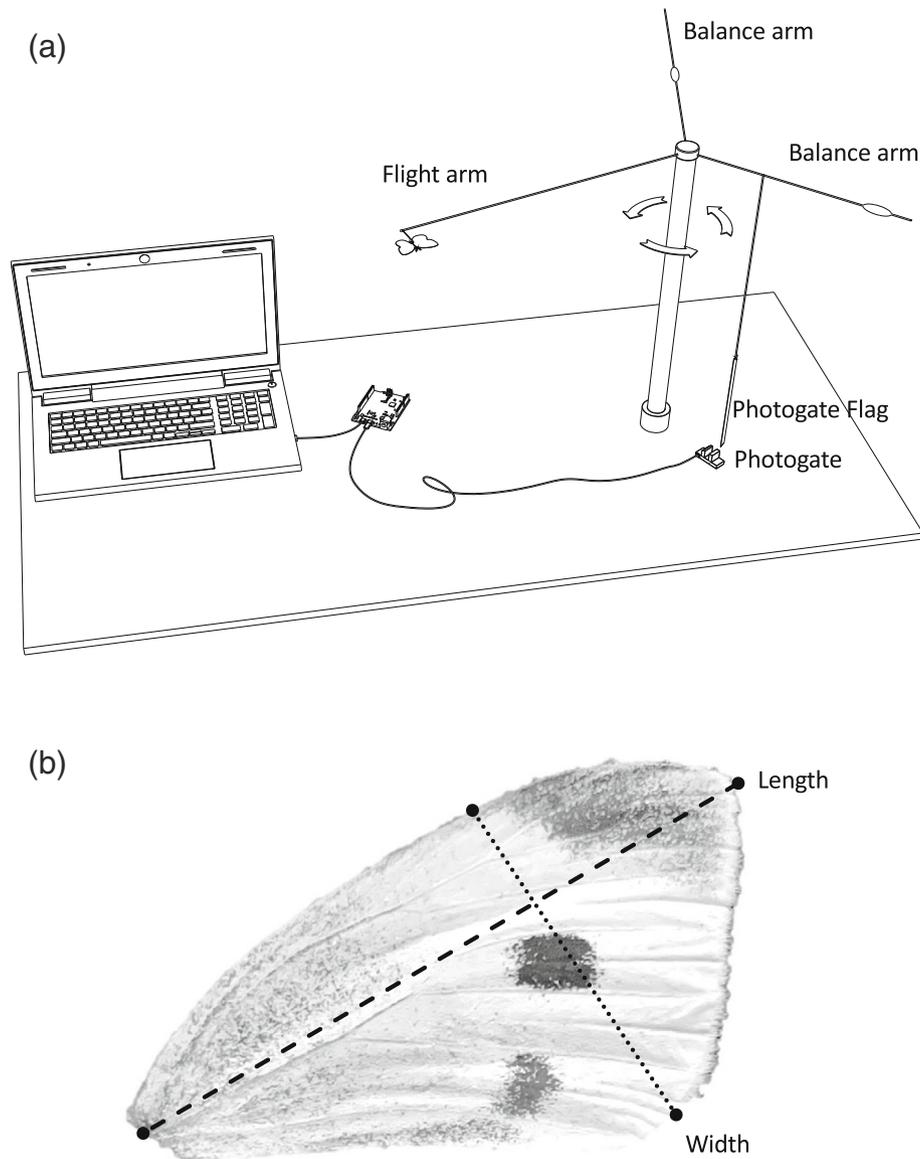


FIGURE 1 Illustrations of the (a) flight mill used in this study and (b) *Pieris rapae* forewing measurements

Columbia campus. This area is approximately 2 km from where the wild-laid eggs were collected in 2020 and thus we consider the wild-laid eggs the wild-caught adults to be part of the same population. The university campus encompasses more than 400 hectares and is a mix of campus buildings and residential housing. The campus houses five community gardens and a 24-hectare working farm. *Pieris rapae* as well as other species of butterflies are highly abundant on campus (MT personal observation). Butterflies were caught using a standard insect net and immediately killed by squeezing the thorax (Lazri & Barrows, 1984). Butterflies were placed in individual 4 oz plastic cups and transported to the laboratory.

Pieris rapae mostly accumulate pollen around their proboscis (Lewis, 1989). We used forceps to gently access the proboscis and palps and we swabbed these areas and nearby sections of the head using a $\sim 1.5 \text{ mm}^3$ block of fuchsin gel (Beattie, 1971). Fuchsin gel blocks were placed on a microscope slide and gently melted on a hot

plate. The number of pollen grains was counted under a light microscope (Zeiss Vert A1). We also quantified pollen richness, which we coarsely define as the number of distinctly different types (in terms of size and shape) of pollen found in the sample. We did not identify pollen grains to species. Similar to the laboratory experiment, we photographed all four wings of these wild-caught individuals and measured wing length, width, and area using ImageJ.

Statistical analyses

Effect of temperature on butterfly life history and wing traits

We used Analysis of Variance (ANOVA, type 3 sums of squares) to investigate whether the three temperature treatments (18°C, 24°C,

30°C) resulted in significant differences in *P. rapae* pupal mass, development time from first instar to adult, total wing area, and wing loading (adult mass/wing area). For pupal mass, development time, and wing area, we combined all data from Experiments 1 and 2. The ANOVA models for these dependent variables included 'Temperature' and 'Experiment' as categorical fixed factors, and the interaction between the two factors. Because we collected adult mass data in Experiment 2 only, the ANOVA for wing loading did not include the 'Experiment' term. We examined whether the data violated assumptions of linear models using histograms of the raw data, plots of fitted values versus residuals, and the Normal Q-Q plot. Pupal mass, development time, and wing loading were left untransformed and wing area was log-transformed. Wing length, wing width, and forewing area were all highly positively correlated to total wing area and thus we used total wing area as our single metric of wing size. If the linear model revealed a significant effect of temperature on life history or morphological traits, we used Tukey HSD post-hoc tests to investigate which pair-wise comparisons were significantly different.

Relationship between total wing area and average velocity

We use a linear model to examine the relationship between total wing area and average flight velocity per revolution. We included 'Experiment' in the model to account for variation due to running two separate experiments. We also included the age (in days) of the adult when it was flown ('Flight Age'). We included this term because it was not always possible to fly the butterfly two days post-eclosion and previous studies have observed effects of age on flight in the closely related *Pieris napi* (Åhman & Karlsson, 2009).

Relationship between total wing area and flight distance

We first visualised the relationship between total wing area and flight distance using a scatterplot. The plot revealed the presence of extreme outliers (defined as a value greater than the 3rd Quartile +3*IQR) and it also showed an apparent non-linear relationship between these two traits. We removed the extreme outliers and included a quadratic term in the model to help account for the

non-linear relationship. The initial model was: $\log(\text{distance}) = \log(\text{total wing area}) + (\log(\text{total wing area}))^2 + \text{Experiment} + \text{Flight Age}$. There was no effect of Flight Age and thus this term was removed.

Relationship between total wing area and pollen metrics of wild-caught butterflies

We used linear models to investigate the relationship between total wing area and pollen count of wild-caught *P. rapae*. Pollen count and wing area were log-transformed to meet assumptions of linear models. The scatterplot between the two traits also revealed a non-linear relationship and thus we included a quadratic term in the model. Because butterflies were caught between early May and mid-June, we also included calendar day in the model. The final model was: $\log(\text{Pollen Count}) \sim \log(\text{total wing area}) + (\log(\text{total wing area}))^2 + \text{Day Caught}$.

Finally, we examined the relationship between pollen count and pollen richness using a linear model. A scatterplot of the two variables revealed a non-linear relationship and thus we included a quadratic term in the model: $\text{pollen richness} \sim \log(\text{pollen count}) + (\log(\text{pollen count}))^2 + \text{Day Caught}$. For these wild-caught butterflies, we used the same methods as describe above (see *Effect of temperature on butterfly life history and wing traits*) to examine whether the data violated assumptions of linear models. All statistical analyses were conducted in R version 4.0.4 (R Core Team, 2021).

RESULTS

Effect of temperature on laboratory-reared butterfly life history and wing traits

There was a significant negative effect of temperature on *P. rapae* pupal mass (Table 1, Figure 2a). Pupae that developed at 18°C were heavier than those reared at 24°C (Tukey HSD $p = 0.012$) or at 30°C (Tukey HSD: $p = 0.0002$). There was no difference in pupal mass between *P. rapae* reared at 24°C versus 30°C (Tukey HSD = 0.7). The effect of temperature on pupal mass also differed slightly between the two experiments. In the 30°C temperature treatment, pupae were

TABLE 1 Results from linear models examining the effect of temperature on *Pieris rapae* pupa mass, development time, wing area, and wing loading

Factor	Pupa mass (mg)	Total development time (days)	Total wing area, log (mm ²)	Wing loading (kg/m ²)
Temperature	$F_{2,95} = 10.9,$ $p < 0.0001$	$F_{2,95} = 1015,$ $p < 0.0001$	$F_{2,95} = 9.1,$ $p = 0.0003$	$F_{2,50} = 5.97,$ $p = 0.005$
Experiment	$F_{1,95} = 0.06,$ $p = 0.8$	$F_{1,95} = 102,$ $p < 0.0001$	$F_{1,95} = 1.4,$ $p = 0.25$	n/a
Temperature x Experiment	$F_{2,95} = 4.1,$ $p = 0.02$	$F_{2,95} = 25.3,$ $p < 0.0001$	$F_{2,95} = 2.88,$ $p = 0.06$	n/a

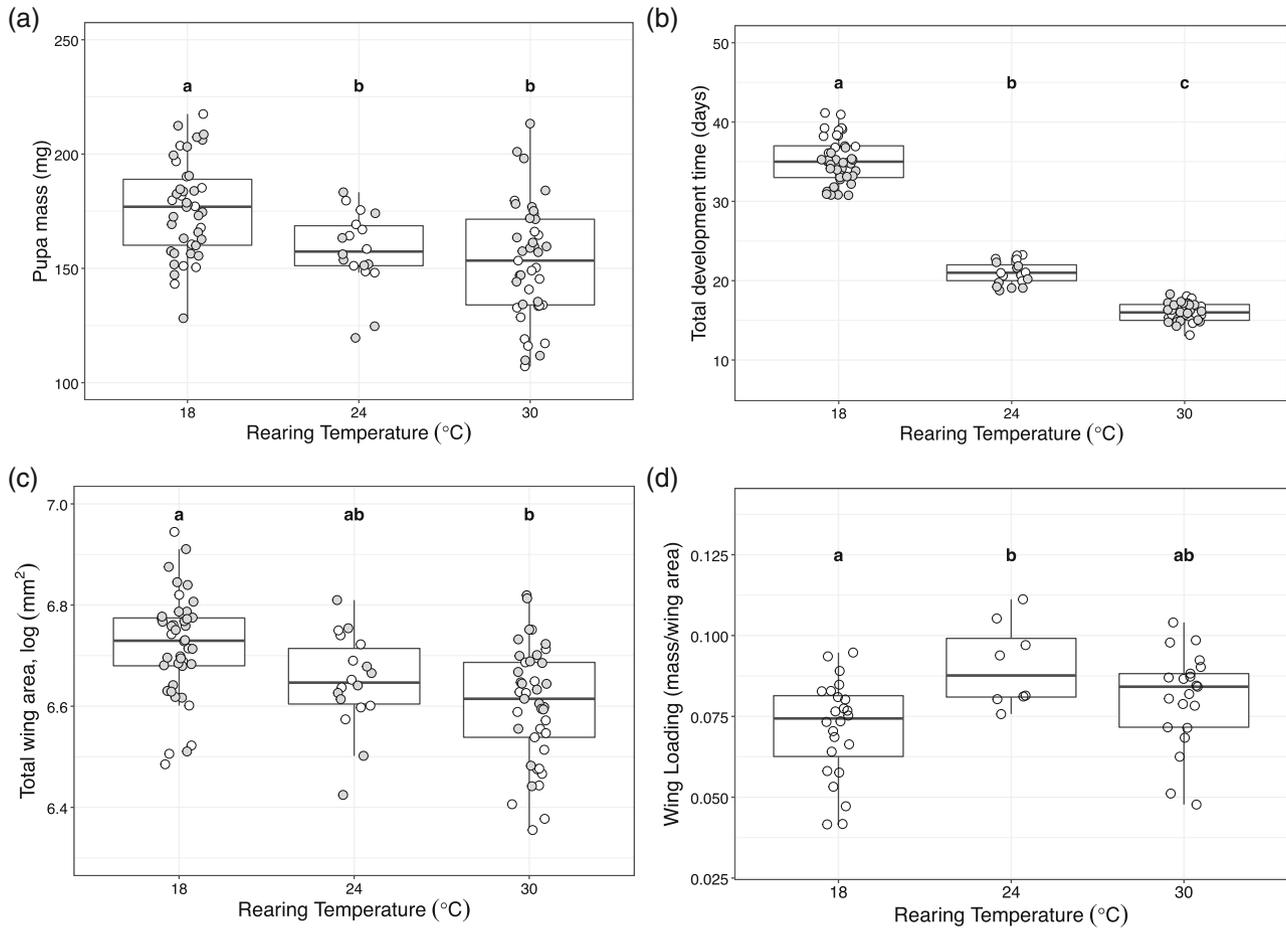


FIGURE 2 Boxplots showing the effect of temperature on *Pieris rapae* (a) pupal mass, (b) total development time, (c) total wing area, and (d) wing loading. White symbols denote data from experiment 1, grey symbols denote data from experiment 2. Letters above the boxplots show which treatments are significantly different according to Tukey HSD tests

12% heavier in Experiment 2 versus Experiment 1 (Table 1, Tukey HSD: $p = 0.03$). Pupal mass did not differ between the experiments in the other two temperature treatments.

Rearing temperature had a significant effect on *P. rapae* development time (Table 1, Figure 2b). Development time was shortened when *P. rapae* were reared at warmer temperatures (Tukey HSD: all pair-wise comparisons $p < 0.0001$). We also observed a slight difference between the two experiments in *P. rapae* development time, with individuals in the 18°C treatment taking 12% less time to develop in Experiment 2 versus Experiment 1 (Tukey HSD: $p < 0.001$). There were no other differences in development time between the two experiments.

Total wing area decreased as rearing temperature increased (Table 1, Figure 2c). The significant effect of temperature was mainly driven by differences in wing area between *P. rapae* grown at 18°C versus 30°C (Figure 2c, Tukey HSD: $p < 0.0001$). Rearing temperature also affected *P. rapae* wing loading (Table 1, Figure 2d). Individuals reared at 18°C had lower mass to wing size ratios compared to those reared at 24°C (Tukey HSD: $p = 0.005$). There was no difference in wing loading between 18°C versus 30°C or between 24°C and 30°C.

Relationship between total wing area and flight metrics

There was a positive relationship between *P. rapae* average flight velocity and total wing area (Figure 3a, $F_{1,97} = 6.52$, $p = 0.012$, full model $r^2 = 0.12$). There was a significant negative effect of flight age (adult age at flight) ($F_{1,97} = 6.15$, $p = 0.01$) on average velocity and no effect of Experiment ($F_{1,97} = 0.07$, $p = 0.79$).

The relationship between total wing area and distance flown was non-linear and marginally non-statistically significant, with butterflies of intermediate size flying slightly longer distances compared to larger or smaller butterflies. (Figure 3b, $F_{1,97} = 3.64$, $p = 0.059$, full model $r^2 = 0.03$). There was no effect of Experiment on distance flown ($F_{1,97} = 2.58$, $p = 0.11$).

Relationship between total wing area and pollen metrics of wild-caught butterflies

The total wing area of *P. rapae* was 5% larger in wild populations compared to those reared in the laboratory (laboratory-reared

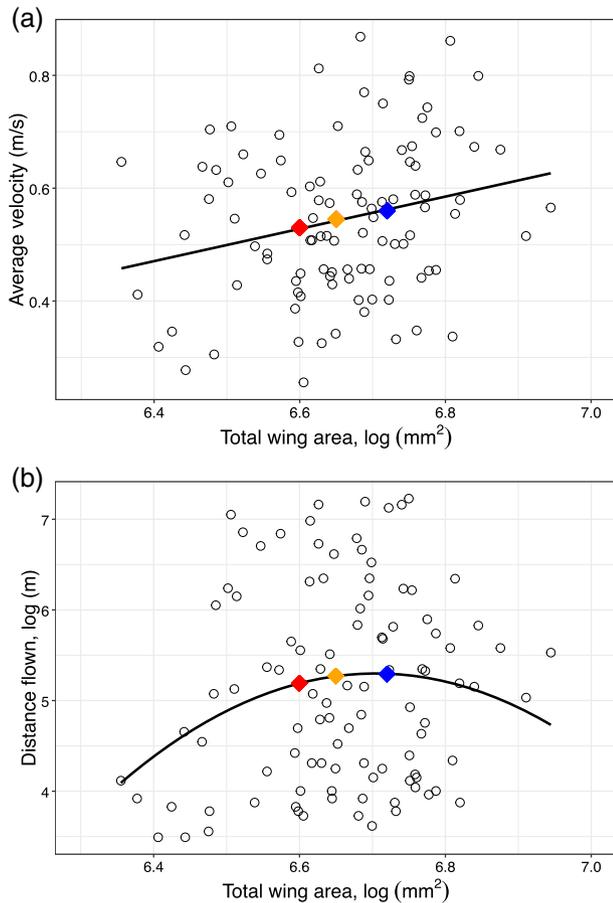


FIGURE 3 Scatterplots of the relationship between total wing area and (a) average velocity ($F_{1,97} = 6.52$, $p = 0.012$), or (b) distance flown ($F_{1,109} = 3.64$, $p = 0.059$) for laboratory-reared *P. rapae*. Coloured symbols denote the mean velocity or distance flown for *P. rapae* grown at 30°C (red), 24°C (orange), and 18°C (blue)

mean \pm standard deviation: $785 \pm 91 \text{ mm}^2$, wild caught: $822 \pm 106 \text{ mm}^2$, Figure S1). There was considerable variation in how much pollen accumulated near the proboscis of wild-caught *P. rapae* (median = 75 pollen grains, mean = 157.8, standard deviation = 180, minimum = 1, maximum = >500).

There was a significant non-linear relationship between total wing area and pollen count (Figure 4a, $F_{1,95} = 4.16$, $p = 0.04$) in wild-caught butterflies. Individuals with wing sizes of approximately 830 mm^2 ($e^{6.72} \text{ mm}^2$) carried the most pollen (~89 pollen grains) and butterflies with wings larger or smaller than 830 mm^2 carried slightly fewer pollen grains. By superimposing mean wing size of lab-reared *P. rapae* (coloured symbols in Figure 4a) onto the relationship between pollen count and total wing area of wild-caught butterflies, we can see that laboratory-reared butterflies that developed at 18°C are very similar in size to the wild-caught butterflies with the highest pollen load (Table 2).

Using our coarse measure of pollen richness, we were able to detect up to five distinct types of pollen grains. We found a significant positive and non-linear relationship between log-pollen count and pollen diversity in wild-caught *P. rapae* (Figure 4b, $F_{1,95} = 6.17$,

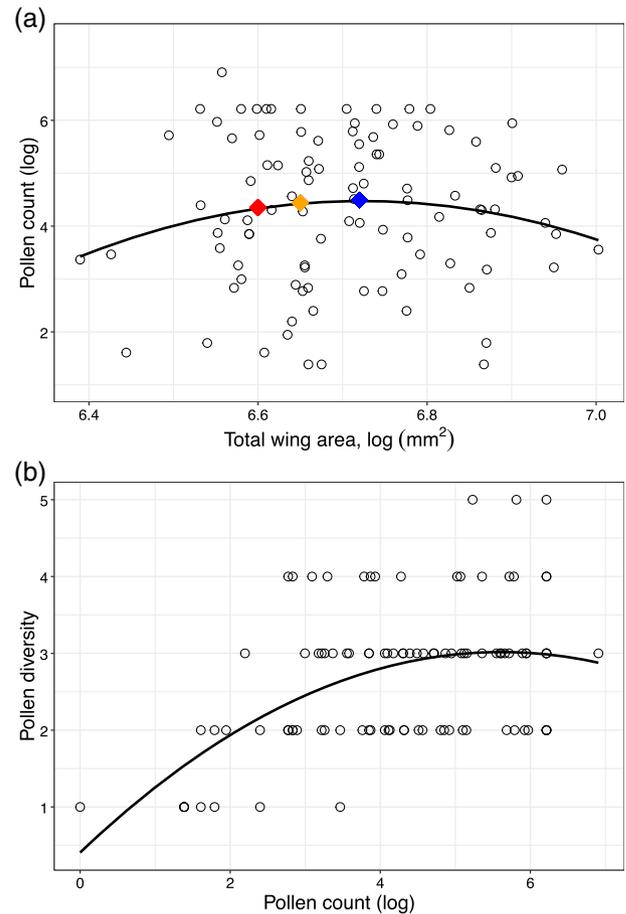


FIGURE 4 Relationship between body size and (a) pollen load ($F_{1,95} = 4.16$, $p = 0.04$), or (b) pollen diversity ($F_{1,95} = 6.17$, $p = 0.015$) in wild-caught *P. rapae*. Coloured symbols denote the estimated pollen count or pollen diversity for wild *P. rapae* of equivalent size to the those reared in the laboratory warming experiment (30°C (red), 24°C (orange), and 18°C (blue))

$p = 0.015$). Pollen diversity generally increased as pollen count increased, but the relationship reached diminishing returns at about 90 ($e^{4.5}$) pollen grains (Figure 4b).

DISCUSSION

While considerable data exists on the effects of temperature on insect body size, and separately on the effects of insect body size on ecological functions, we have limited evidence that directly links temperature-mediated changes in insect body size to community-level consequences in nature. Concomitantly, we have little data that demonstrates the applicability of results from laboratory warming experiments to wild populations. Given the hundreds of laboratory warming experiments that have been published recently, a major gap in our understanding of ecological responses to warming is knowing how well laboratory studies predict patterns in nature. Here, we used a laboratory experiment to examine the effects of warming temperatures on body size and flight in *P. rapae*, and we tested the relevance of

TABLE 2 Linking laboratory body size and flight data to pollen collection in nature

Temp. (°C)	Total wing size (mm ²)	Average velocity (m/s)	Distance flown (m)	Pollen count from wild-caught of equivalent size	Pollen richness from wild-caught individuals
18	832	0.56	200	89	2.92
24	775 (–7%)	0.545 (–3%)	190 (–5%)	84 (–6%)	2.90
30	742 (–5%, –11%)	0.53 (–3%, –5%)	172 (–10%, –14%)	77 (–8%, –14%)	2.89

Notes: Wing size, average velocity, and distance flown are data from *Pieris rapae* grown in the laboratory experiment at 18°C, 24°C, or 30°C. Pollen count is estimated using the non-linear relationship between wing area and pollen count for wild-caught *P. rapae* (Figure 4a). Pollen richness is estimated using the non-linear relationship between pollen count and pollen richness (Figure 4b). The percentages in parentheses reflect the percent decrease in value relative to butterflies reared at the next coldest temperature.

these results in nature by characterising pollen load in wild *P. rapae* of similar size to those generated in the laboratory experiment.

Overall, we found that butterflies were smaller when reared in the laboratory at warmer temperatures, and when tested on a flight mill, smaller butterflies flew more slowly and tended to fly shorter distances compared to larger butterflies (Table 2). We predicted that in nature, smaller butterflies with reduced flight capacity may end up visiting fewer flowers and amassing less pollen. Indeed, when we examined the relationship between wing size and pollen number of wild-caught butterflies, wild butterflies of equivalent size to the 30°C laboratory-reared butterflies carried 8% and 14% less pollen than did wild butterflies of equivalent size to the 24°C or 18°C-reared laboratory butterflies, respectively (Table 2). Our study thus demonstrates that developmental temperature has predictable effects on *P. rapae* body size and flight, and that even subtle decreases in wing size in nature have measurable effects on flower visitation and pollen accumulation.

The pupa mass, development time, and wing size data observed here were all consistent with other studies that have examined the effects of temperature on *P. rapae* development and morphology (Jones et al., 1982; Ohsaki, 1986; Seiter et al., 2013; Seiter & Kingsolver, 2013). The response of *P. rapae* to warming is population-specific (Kingsolver et al., 2006; Lin et al., 2018; Seiter & Kingsolver, 2013), and here the results from both experiments were as expected for *P. rapae* from temperate populations. Our data for pollen load and diversity are also within the range found in this species (Lazri & Barrows, 1984). The effect of temperature on pupal mass and total development time differed slightly between the two experiments. These differences were perhaps expected given known differences in *P. rapae* growth and development among cohorts within the temperate growing season (Kimura & Tsubaki, 1986).

To the best of our knowledge, *P. rapae* have not been assayed on flight mills before and thus we have no studies to which compare our flight data. However, we have confidence in the results because: (a) the data were consistent across two separately run experiments, (b) the positive relationship between wing size and flight speed has also been documented in other species (Framout et al., 2018; Jahant-Miller et al., 2022), and (c) *P. rapae* reared at the coldest temperature (18°C) had lower wing loading and faster flight speed compared to those reared at 24°C or 30°C. Lower mass to wing size ratios were also associated with improved dispersal and faster take-off speed in

other species of *Pieris* (Almbro & Kullberg, 2012; Fric et al., 2006). Together, the repeatability of the flight data, combined with parallel changes in *P. rapae* morphological traits, suggest that the flight data were valid and not an artefact of our custom flight mill. The results from this experiment are also similar to studies conducted on the relationship between insect body size and flight in other species. For example, monarchs reared at 25°C flew longer and farther compared to monarchs reared at 28°C (Soule et al., 2020). Whether flight ability was also correlated with monarch body size was not discussed in the paper. Additionally, a 4°C increase in rearing temperature resulted in reduced flight muscle mass and flight endurance in the damselfly *Ishnura elegans* (Tüzün et al., 2018).

Although flight mills are commonly used to assess insect flight (Naranjo, 2019), it is unclear how data acquired from flight mills translate into performance in nature. Flight mill data may overestimate the average flight period of any given insect because on the flight mill, flight typically stops when the insect is exhausted, whereas in nature, insects will stop flying for a number of different reasons (Gatehouse & Hackett, 1980). We believe that our data on wild-caught butterflies of approximately equivalent size to laboratory-reared *P. rapae* help to assuage the notion that the flight mill data are not particularly relevant to natural settings. Given that smaller butterflies flew less quickly and slightly less far in the laboratory, we expected smaller wild-caught butterflies also to carry less pollen. Indeed, this was the pattern we observed from our sample of 100 wild-caught *P. rapae*. However, insect body size in nature can also be affected by food quality and quantity, and thus we cannot rule out that the wing size-pollen abundance relationship was not caused by factors that were not measured here. It is also possible that wing size is one of many traits that affect pollen accumulation, and that laboratory-reared versus wild-caught *P. rapae* may differ in key pollen-collecting traits that we did not measure. Future studies that examine mechanistically why smaller butterflies carry less pollen, or that examine the key determinates of variation in insect body size in nature are needed to better understand the relationship between insect body size and plant-insect interactions.

Overall, the results of this study provide insight into how body size and flight ability can be predicted by the developmental temperature of the butterfly, and into the potential broader consequences of temperature-mediate size variation for plant-pollinator interactions. We were also able to fairly accurately predict pollen collection

behaviour in nature based on the results of a simple laboratory warming experiment. A growing number of studies have reported decreases in insect body size in response to climate warming (Kelemen & Rehan, 2021; Tseng et al., 2018; Wilson et al., 2019; Wonglersak et al., 2021). Unfortunately, we currently have too little data to predict the general effects of shrinking insects on the many ecological functions and ecosystem services performed by this specious animal group. Given the widespread importance of insects, we believe there is a pressing need to continue quantifying the multiple ways that climate-mediated decreases in insect body size may alter the functioning of ecological communities.

AUTHOR CONTRIBUTION

Erez Büyükyılmaz and Michelle Tseng designed the study; EB conducted the study and collected the data; Erez Büyükyılmaz and Michelle Tseng conducted the analyses and wrote the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in dryad at <https://datadryad.org/stash>.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure S1 Boxplots showing wing area distributions for laboratory-reared and wild-caught *Pieris rapae*.

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