

RESEARCH ARTICLE

Irreversible impact of early thermal conditions: an integrative study of developmental plasticity linked to mobility in a butterfly species

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ABSTRACT

Within populations, phenotypic plasticity may allow adaptive phenotypic variation in response to selection generated by environmental heterogeneity. For instance, in multivoltine species, seasonal changes between and within generations may trigger morphological and physiological variation enhancing fitness under different environmental conditions. These seasonal changes may irreversibly affect adult phenotypes when experienced during development. Yet, the irreversible effects of developmental plasticity on adult morphology have rarely been linked to life-history traits even though they may affect different fitness components such as reproduction, mobility and self-maintenance. To address this issue, we raised larvae of *Pieris napi* butterflies under warm or cool conditions to subsequently compare adult performance in terms of reproduction performance (as assessed through fecundity), displacement capacity (as assessed through flight propensity and endurance) and self-maintenance (as assessed through the measurement of oxidative markers). As expected in ectotherms, individuals developed faster under warm conditions and were smaller than individuals developing under cool conditions. They also had more slender wings and showed a higher wing surface ratio. These morphological differences were associated with changes in the reproductive and flight performance of adults, as individuals developing under warm conditions laid fewer eggs and flew larger distances. Accordingly, the examination of their oxidative status suggested that individuals developing under warm conditions invested more strongly into self-maintenance than individuals developing under cool conditions (possibly at the expense of reproduction). Overall, our results indicate that developmental conditions have long-term consequences on several adult traits in butterflies. This plasticity probably acts on life-history strategies for each generation to keep pace with seasonal variations and may facilitate acclimation processes in the context of climate change.

KEY WORDS: Seasonality, Flight performance, Life-history patterns

INTRODUCTION


The concept of life-history theory predicts how individuals can allocate limited resources between competing functions (e.g. growth, maintenance and reproduction; Stearns, 1976). In nature, co-adapted sets of traits, improving fitness components in a specific environment, define life-history patterns (Alonzo et al., 2008). In multivoltine species, seasonal polyphenism (spring or summer morphs: Karlsson and Johansson, 2008) has been correlated with life-history patterns; for example, depending on the developmental pathways (direct or diapause: Larsdotter Mellström et al., 2010). Environmental cues such as temperature, light and humidity may act as predictors for future selection and induce the emergence of different phenotypes (Chevin and Lande, 2015). In insects inhabiting seasonal environments, temporal variation in environmental conditions throughout years can promote the evolution of plastic morphology and/or physiology through developmental plasticity, matching environmental contexts subsequently encountered during adulthood (Halali et al., 2021; Nettle and Bateson, 2015). However, the irreversible impacts of this developmental plasticity on the adult phenotype may durably affect the regulation of trade-offs between functions and therefore play a role in the evolution of life-history traits (Berger et al., 2012; Klockmann et al., 2017; Van Dyck and Holveck, 2016). For example, in holometabolous species with distinct life stages (e.g. larvae, pupae, adults), high temperatures accelerate the development of pre-adult individuals (Chown and Nicolson, 2004; Nedved, 2009; Régnière et al., 2012), resulting in smaller and lighter adult individuals (temperature size rule; Ratte, 1984). Investigating the impact of developmental plasticity at the individual level could help uncover the mechanisms behind the process of acclimatization to seasonal changes.

To examine the costs and benefits of developmental plasticity and the impact on acclimatization processes, several parameters need to be considered. For instance, the fitness of butterflies does not only depend on the number of eggs they lay or their longevity but also on their ability to fly. Indeed, flight is intrinsically related to survival, as it is essential for different activities such as foraging and escaping predators. It is also related to reproduction, as it is involved in sex-specific activities such as mate location, courtship and patrolling for males, and host plant selection for females. Flight is therefore an important determinant of the mating strategy and reproductive success of butterflies (Rutowski, 1998). Flight components, such as morphology and behaviour, are probably interrelated as they may have evolved under common selective pressures (Le Roy et al., 2019b) but also depend on the interaction between the internal state of the individual and the external conditions it experiences (Chaput-Bardy et al., 2010). Internal individual state, referring to the time-condition dependent status of the individual, might explain ultimate behavioural decisions such as fight or flight responses (Stevenson and Rillich, 2012). Correlations between movement capacity and

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movement propensity depending on internal individual state (e.g. morphology, metabolic rate, oxidative status) can therefore be examined by controlling the environmental conditions experienced by individuals.

In response to environmental heterogeneity, organisms may alter how they regulate their balance between self-maintenance and reproduction through phenotypic plasticity, thereby improving fitness and/or fecundity output in the different environments encountered. For instance, self-maintenance mechanisms, such as antioxidant defences, may be enhanced in response to limited availability of resources at the expense of other traits (e.g. reproduction, displacement; Jenni-Eiermann et al., 2014; Metcalfe and Monaghan, 2013). Hence, the oxidative status (i.e. the balance between antioxidant defences and oxidative damage) may be a key mechanism mediating life-history trade-offs (Monaghan et al., 2009), such as that between flight-related traits and reproduction.

To unravel the potential impact of developmental temperatures on life-history patterns in *P. napi* butterflies, we raised siblings under contrasted thermal conditions at larval and pupal stages to subsequently examine their performance at adulthood under intermediate thermal conditions. Specifically, we focused on the variation of life-history traits which may highlight how resources are allocated between functions (e.g. self-maintenance versus flight-related traits: Costantini et al., 2008, 2018; Jenni-Eiermann et al., 2014; reproduction versus self-maintenance: Beaulieu et al., 2015; and flight-related traits versus reproduction: Gibbs and van Dyck, 2010). With this integrative approach, we aimed to assess whether a durable change in morphology and physiology due to plasticity triggered by developmental temperatures could give rise to alternative life-history patterns (Karlsson and Wiklund, 2005). In the wild, the temperatures experienced during development may reliably indicate the future conditions encountered by adults (Nettle and Bateson, 2015). For instance, hot developmental conditions might be a cue to fragmented resource availability encountered at adulthood (Holyoak and Heath, 2016; Piessens et al., 2009). We therefore predicted that abnormally warm developmental conditions may signal poor and stressful environmental conditions that adult individuals may subsequently have to resist (by activating self-maintenance mechanisms: Günter et al., 2020a) or to escape (by enhancing flight performance: Battisti et al., 2006; Kuussaari et al., 2016). Allocating resources to self-maintenance and dispersal capacity may, however, come at the cost of a lower reproductive performance (Stearns, 1989). We therefore expected individuals developing under warm conditions to lean towards a more dispersive life-history pattern, by enhancing self-maintenance and flight-related traits over direct fecundity output.

MATERIALS AND METHODS

Biological model and collection

The green-veined white butterfly *Pieris napi* (Linnaeus 1758) (Lepidoptera: Pieridae) is a common and widespread butterfly species, present in almost all European countries and the temperate zone of Asia. Depending on latitude and altitude, the flight season ranges from March to October, with up to four generations per year experiencing different conditions of temperature, humidity, photoperiod, food and host plant availability (Petersen, 1949). Adults feed on a wide range of flowering plants including those from the Lythraceae family, while larvae feed exclusively on plants from the Brassicaceae family.

For our study, 25 females of the second generation were caught in June 2018 in the surroundings of Greifswald, Germany (54°08'N, 13°37'E). Females were placed individually into translucent 1 litre plastic pots covered with gauze in a climate chamber set at 21.5°C, 60% humidity, and a 20 h:4 h light:dark photoperiod. During the following 8 days, each female was provided with *Alliaria petiolate* for oviposition, fresh flowers (*Achillea millefolium*, *Leucanthemum vulgare*, *Hypochaeris radicata*) and a sucrose solution (20% volume in water) for feeding. Deposited eggs were collected daily and transferred to small plastic boxes (4×10×6 cm). For each female, resulting larvae were randomly divided into two treatment groups experiencing different developmental temperatures (split-brood design).

Rearing experiment

The first group of larvae ($N=1679$) was reared under cool conditions (18°C, 60% humidity, 20 h:4 h light:dark photoperiod), while the second group ($N=1371$) experienced warm conditions (25°C, 60% relative humidity, 20 h:4 h light:dark photoperiod; see Fig. 1); 18°C corresponds to the mean temperature that butterflies experience in their natural habitat in North-East Germany in July while 25°C reflects abnormally high temperatures (Günter et al., 2020b). Exposure to constant temperatures does not reflect natural conditions but represents a useful tool to investigate phenotypic plasticity (Fischer et al., 2011). Even though the light duration may be considered long (ca. daylight and civil twilight on summer solstice in Northern Germany), the photoperiod was chosen to prevent individuals from entering diapause during the experiment (Beck, 1980; Zaslavski, 1988) and was constant in the two groups (Gotthard, 2004; von Schmalensee et al., 2021).

Larvae were reared individually in small plastic boxes (4×10×6 cm) with moistened filter paper and had *ad libitum* access to fresh cuttings of *Brassica napus oleifera* until pupation. Pupae were weighed on the day following pupation. For each individual, we recorded larval and pupal development time and mortality at each developmental stage (Fig. 2A). Hatching rate (%)

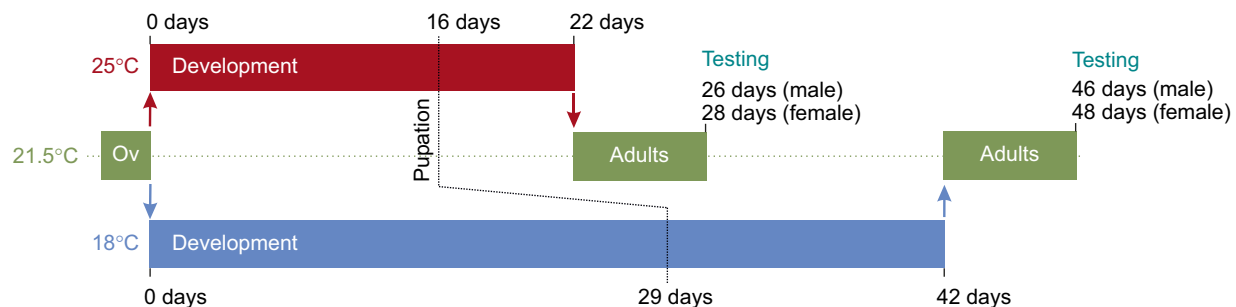


Fig. 1. Chronogram of the experiment. Oviposition (Ov) represents egg laying of the wild-caught individuals, F0.

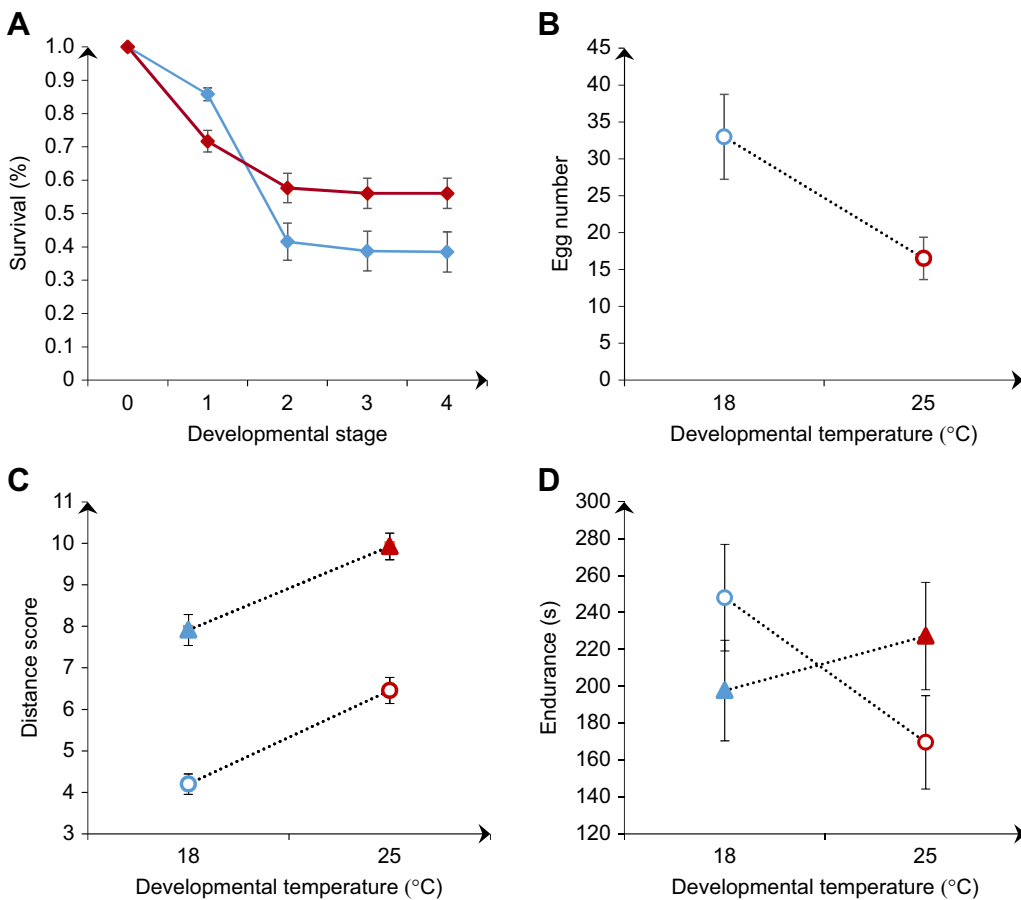


Fig. 2. Impact of developmental conditions on survival, fecundity and flight performance. (A) Kaplan–Meier survival curve across developmental stages (0: eggs, 1: hatching, 2: larval stage, 3: pupal stage, 4: eclosion) and mean \pm s.e.m. (B) egg number, (C) flight distance and (D) flight endurance in relation to developmental temperature and sex (the latter for C and D only). Developmental temperature is represented by colour (blue: 18°C, red: 25°C) and sex by shape (diamonds: males and females, circles: females, triangles: males).

was calculated by dividing the number of larvae by the number of collected eggs per female $\times 100$. After eclosion, all adult butterflies were identified with a unique number written on the right dorsal forewing and kept at an intermediate temperature (21.5°C, 60% humidity, 20 h:4 h light:dark photoperiod). They were isolated by temperature treatment and sex in hanging nets with sugar solution and fresh flowers (as above). To account for the protandry occurring in nature, 1 day old females and 2 day old males were then put together in a net for mating each day from 10:00 h to 17:00 h. Pairs of mated butterflies were immediately placed into individual translucent 1 litre plastic pots covered with a gauze. After mating, males and females were separated and placed in individual 1 litre plastic pots. They were provided with fresh flowers (*Achillea millefolium*, *Leucanthemum vulgare*, *Hypochaeris radicata*) and a sucrose solution for feeding. In addition, mated females were provided with *Alliaria petiolate* for oviposition, and deposited eggs were counted daily to estimate early fecundity output. Mated males were tested for flight when they were 4–5 days old while mated females were allowed to oviposit for 6–7 days before flight measurements (females: $N_{18^\circ\text{C}}=35$, $N_{25^\circ\text{C}}=46$; males: $N_{18^\circ\text{C}}=34$, $N_{25^\circ\text{C}}=43$).

Flight performance

We followed the protocol described by Ducatez et al. (2012) to estimate the propensity of butterflies to fly under unfamiliar conditions. Specifically, in a dark room held at a constant temperature of 21.5 \pm 1°C, butterflies were individually released at the entrance of a 3 m long opaque pipe with an 80 cm diameter and with a light bulb (Zoo Med Powersun UV Self-Ballasted Mercury Vapor UVB Lamp 160 W) reproducing sunlight spectrum at its end.

This test was previously used to estimate the bold/shy tendency of a closely related species (*Pieris brassicae*) by scoring the butterflies which flew into the pipe (bold) and those that did not (shy; Ducatez et al., 2012). As all our butterflies flew into the pipe, we decided to measure how far each butterfly flew into the tunnel before landing and staying in place for more than 5 s. Three consecutive trials were recorded and a score was attributed for the flight distance inside the pipe (0–50 cm=1; 51–100 cm=2; 101–150 cm=3; 151–200 cm=4; 201–250 cm=5; 251–300 cm=6). The addition of the scores obtained in the three trials allowed us to reduce intra-individual variation and represented a proxy for the behavioural propensity of butterflies to fly.

To decouple the propensity and the capacity to fly under stressful conditions, butterflies were tested following the tunnel test, for flight endurance in a climate chamber at 21.5°C and 60% humidity. We measured metabolic rate simultaneously during this test to examine whether flight energetic requirements differed between our two experimental groups. Specifically, each individual was kept in a custom-designed sealed 1 litre plastic chamber coupled with a flow-through respirometry system (Q-bit system, Q-S151 CO₂ Analyzer, Kingston, ON, Canada). The CO₂ production was recorded with ‘Logger Pro’ software (v.3.15, Vernier, Beaverton, OR, USA) with dried air and standardized CO₂ air gas (500 ppm) flowing through at a rate of 50–60 ml min⁻¹. After acclimation within the plastic chamber for 10 min, butterflies were forced to fly by strongly shaking the chamber using a rapid agitator (IKA Vortex 4 digital), thus preventing them from holding on to the walls of the plastic chamber (Ducatez et al., 2012). The time an individual butterfly spent flying and the peak of CO₂ production were recorded. Peak CO₂ production was then divided by the time spent in flight to

estimate maximum flight metabolic rate (Mitikka and Hanski, 2009). After 24 h of recovery, butterflies were frozen on dry ice and stored at -80°C for further morphological and physiological analyses. This recovery time was necessary for the measurement of physiological parameters to reflect basal levels (i.e. not reflecting flight effects).

Morphological analyses

Morphological parameters were measured after mating and flight tests and were used as potential explanatory variables for flight performance. Flight performance may be influenced by body mass and wing morphology (Le Roy et al., 2019a). However, in contrast to body mass, which may fluctuate at adulthood as a result of inter-individual differences in feeding behaviour or fecundity, wing morphology is irreversibly determined during ontogenesis and reflects the direct effects of developmental temperature on adult body size (Frankino et al., 2007). First, total adult body mass was determined to the nearest 0.01 mg using a fine scale (Sartorius CPA225D). Then, wings, heads and legs were removed on dry ice. Thorax and abdomen were separated and afterwards weighed independently (± 0.01 mg). The forewings and hindwings (dorsal and ventral side) were photographed under standardized light conditions with a digital camera (Canon EOS 500D equipped with an Ultrasonic 300 mm lens) mounted on a focal-plane adjustable stand. Wing area and length (from basis to apex) were measured using the lasso tool in Adobe Photoshop CS (Adobe, Inc., San José, CA, USA). Wing loading was calculated as total body mass divided by wing area (forewing+hindwing), and wing aspect ratio as $(4 \times \text{wing length}^2) / \text{wing area}$ to describe wing shape (with higher values reflecting more slender wings; Betts and Wootton, 1988). Relative thorax mass was calculated by dividing thorax mass by total mass (a proxy reflecting investment in thorax flight muscles). Because the mass of butterflies was measured after they reproduced and participated in flight tests, we caution that these values may not exactly reflect their mass at eclosion, as reproduction and flight investment may both affect this parameter.

Oxidative status

A subset of individuals of both sexes ($N_{18^{\circ}\text{C}}=32$; $N_{25^{\circ}\text{C}}=31$) was chosen to examine whether butterflies differentially invested resources in self-maintenance (as assessed through the measurement of markers of oxidative status) depending on developmental temperature. Toward this end, thoraces were first transferred into tubes filled with 500 μl of phosphate-buffered saline (PBS: 100 mmol l^{-1} , pH 7.4) and metal beads (3 mm) to crush tissues through high-speed shaking (Mixer Mill MM400; 30 s at 30 Hz; Retsch, Eragny Sur Oise, France). Samples were then centrifuged (10,000 g , 10 min, 4°C). Finally, the resulting supernatant was collected and transferred to a clean tube. Three markers of oxidative status were measured. The total antioxidant capacity of supernatants was measured after dilution (1:10) with the OXY-adsorbent test (Diacron International, Grosseto, Italy; reading at 510 nm with a spectrophotometer, Tecan i-control 1.5 14.0, infinite 200). A superoxide dismutase (SOD) determination kit (Sigma Aldrich Chemie, Buchs, Switzerland) was used to measure SOD activity, a prime antioxidant enzyme that catalyses the dismutation of the superoxide anion into hydrogen peroxide. Finally, a DNA Damage (8-OHdG) ELISA kit (StressMarq, Victoria, BC, Canada) was used to measure 8-hydroxy-2-deoxy guanosine, which is produced through the action of reactive oxygen and nitrogen species on DNA. The protein content of supernatants

was then measured using the Bradford method to standardize oxidative parameters among individuals.

Statistical analyses

The Kaplan–Meier method from the R package ‘survival’ (Therneau and Grambsch, 2000) was used to compare survival curves between developmental groups. The package ‘lme4’ (Table S4) was used to perform linear mixed effects analyses on flight performance, developmental, morphological and physiological traits with developmental temperature, sex and the interaction between the two terms as fixed effects. As several individuals were siblings, family was added as a random factor in the models. Visual inspection of residual qqplots did not reveal any obvious deviation from homoscedasticity or normality. P -values were obtained by likelihood ratio tests (REML) of the full model with the considered effect tested against the model without it. The P -values associated with the random effect were obtained using the rand function from the ‘lmerTest’ package (Kuznetsova et al., 2017). For each linear mixed model, the package ‘emmeans’ (Table S4) was used to obtain the pairwise comparisons between sex \times developmental temperature groups. The same analysis was conducted to compare the fecundity (i.e. total number of eggs) of females developing under cool or warm conditions, with developmental temperature as a fixed effect and family as a random factor. Comparisons between experimental groups were conducted for oxidative markers using general linear models, as these parameters were only measured in a subset of individuals equally distributed between developmental temperatures and sexes.

Based on all morphological parameters (Table 1), a principal component analysis (PCA, package ‘factoextra’; Table S4) was performed for each experimental group, to reduce the number of morphological parameters characterizing each individual. Eigen values between 4.2 and 1.2 for females and 4.7 and 1.1 for males were used. We then used the scores of the first four principal components coordinates in ANCOVA analyses with flight parameters (endurance and distance) as dependent variables to examine correlations between morphology and flight performances. We refrained from examining relationships between flight parameters and oxidative markers, as our sample size was too small for such analyses.

The results are provided as means \pm s.e.m. for each experimental group in Table S1. All statistical analyses were performed in R (<http://www.R-project.org/>).

RESULTS

Impact of developmental conditions on survival, life-history traits and ultimate adult morphology

Survival was significantly affected by developmental temperature, with individuals developing under warm conditions having an overall higher survival probability than individuals developing under cool conditions (Table 1, Fig. 2A). Moreover, compared with individuals raised under warm conditions, individuals raised under cool conditions showed longer larval (+13 days) and pupal times (+6 days), higher pupal (+2 mg), adult (+8 mg) and thorax mass (+3 mg), larger wing area and length, rounder forewings and a higher wing surface ratio (Table S1; Fig. 3). Pupal and adult mass were positively correlated within each experimental group (Fig. S1), suggesting that adult mass was largely determined during development. Developmental temperature significantly affected all traits measured except abdomen mass, weight loss, relative thorax mass and wing loading (Table 1). Importantly, mass differences between adult butterflies raised under cool and warm conditions

Table 1. Results of general and generalized linear mixed effects models with developmental temperature, sex and their interaction as fixed terms and family as random factor

		Developmental temperature	Sex	Developmental temperature×sex	Family (random)	
Survival	Overall survival	$\chi^2_1=4.6$, $P=0.032$	–	–	–	
Fecundity	Number of eggs	$F_{1,80}=2.77$, $P=0.007$	–	–	$\chi^2_1=1.6$, $P=0.21$	
Flight	Distance	$\chi^2_2=40.1$, $P<0.001$	$\chi^2_2=94.2$, $P<0.001$	$\chi^2_1=0.1$, $P=0.71$	$\chi^2_1=1.2$, $P=0.27$	
	Endurance	$\chi^2_2=5.8$, $P=0.055^*$	$\chi^2_2=5$, $P=0.084^*$	$\chi^2_1=4.6$, $P=0.032$	$\chi^2_1=5.3$, $P=0.021$	
Development	Larval time	$\chi^2_2=488.1$, $P<0.001$	$\chi^2_2=0.9$, $P=0.64$	$\chi^2_1=0.9$, $P=0.64$	$\chi^2_1=29.3$, $P<0.001$	
	Pupal time	$\chi^2_2=439.8$, $P<0.001$	$\chi^2_2=5.2$, $P=0.08$	$\chi^2_1=1.7$, $P=0.19$	$\chi^2_1=1$, $P=0.31$	
	Pupal mass	$\chi^2_2=49.1$, $P<0.001$	$\chi^2_2=29.5$, $P<0.001$	$\chi^2_1=0.3$, $P=0.56$	$\chi^2_1=17.4$, $P<0.001$	
	Weight loss	$\chi^2_2=0.05$, $P=0.98$	$\chi^2_2=50.5$, $P<0.001$	$\chi^2_1=0.05$, $P=0.83$	$\chi^2_1=0.5$, $P=0.48$	
	Abdomen mass	$\chi^2_2=5.2$, $P=0.07$	$\chi^2_2=36.9$, $P<0.001$	$\chi^2_1=0.1$, $P=0.74$	$\chi^2_1=0.7$, $P=0.40$	
	Thorax mass	$\chi^2_2=33.8$, $P<0.001$	$\chi^2_2=16.7$, $P<0.001$	$\chi^2_1=0.3$, $P=0.60$	$\chi^2_1=0.2$, $P=0.64$	
	Relative thorax mass	$\chi^2_2=2.5$, $P=0.29$	$\chi^2_2=90$, $P<0.001$	$\chi^2_1=0.2$, $P=0.67$	–	
	Wing area	$\chi^2_2=32.2$, $P<0.001$	$\chi^2_2=20.3$, $P<0.001$	$\chi^2_1=0.1$, $P=0.7163$	$\chi^2_1=0.1$, $P=0.26$	
	Forewing length	$\chi^2_2=30$, $P<0.001$	$\chi^2_2=31.7$, $P<0.001$	$\chi^2_1=0.01$, $P=0.95$	$\chi^2_1=0.5$, $P=0.23$	
	Wing loading	$\chi^2_2=2.4$, $P=0.50$	$\chi^2_2=33.5$, $P<0.001$	$\chi^2_1=0.4$, $P=0.71$	$\chi^2_1=6.7$, $P=0.009$	
	Forewing aspect ratio	$\chi^2_2=1.6$, $P=0.024$	$\chi^2_2=8.6$, $P<0.001$	$\chi^2_1=0.3$, $P=0.48$	$\chi^2_1=0.01$, $P=0.93$	
	Wing surface ratio	$\chi^2_2=27.4$, $P<0.001$	$\chi^2_2=14.4$, $P<0.001$	$\chi^2_1=1.9$, $P=0.17$	$\chi^2_1=0.1$, $P=0.12$	
	Physiology	Antioxidant capacity	$F_{1,439}=8.03$, $P=0.007$	$F_{1,69}=1.26$, $P=0.27$	$F_{1,122}=2.23$, $P=0.14$	d.f.=59, MS=54.6
		Superoxide dismutase	$F_{1,313}=14.9$, $P<0.001$	$F_{1,40}=1.91$, $P=0.17$	$F_{1,43}=2.05$, $P=0.16$	d.f.=59, MS=20.96
DNA damage		$F_{1,4161}=21$, $P<0.001$	$F_{1,5368}=0.27$, $P=0.61$	$F_{1,2119}=1$, $P=0.31$	d.f.=59, MS=2028	
Flight metabolic rate		$\chi^2_2=2.6$, $P=0.27$	$\chi^2_2=6.1$, $P=0.047$	$\chi^2_1=1.3$, $P=0.25$	$\chi^2_1=0.7$, $P=0.41$	

Significant P -values ($P<0.05$) are shown in bold and statistical tendencies ($P<0.10$) are marked with as asterisk.

may have been minimized in our study, as we weighed them when they had already reproduced (and heavier females raised under cool conditions had already laid more eggs than lighter females raised under warm conditions). Significant sexual differences prevailed in all traits measured except larval and pupal development time and

hindwing aspect ratio (Table 1): females showed higher pupal but lower thorax mass, smaller and rounder wings, higher wing loading, and lower wing surface ratio than males (Fig. 3). The interaction between sex and developmental temperature was not significant for any parameter.

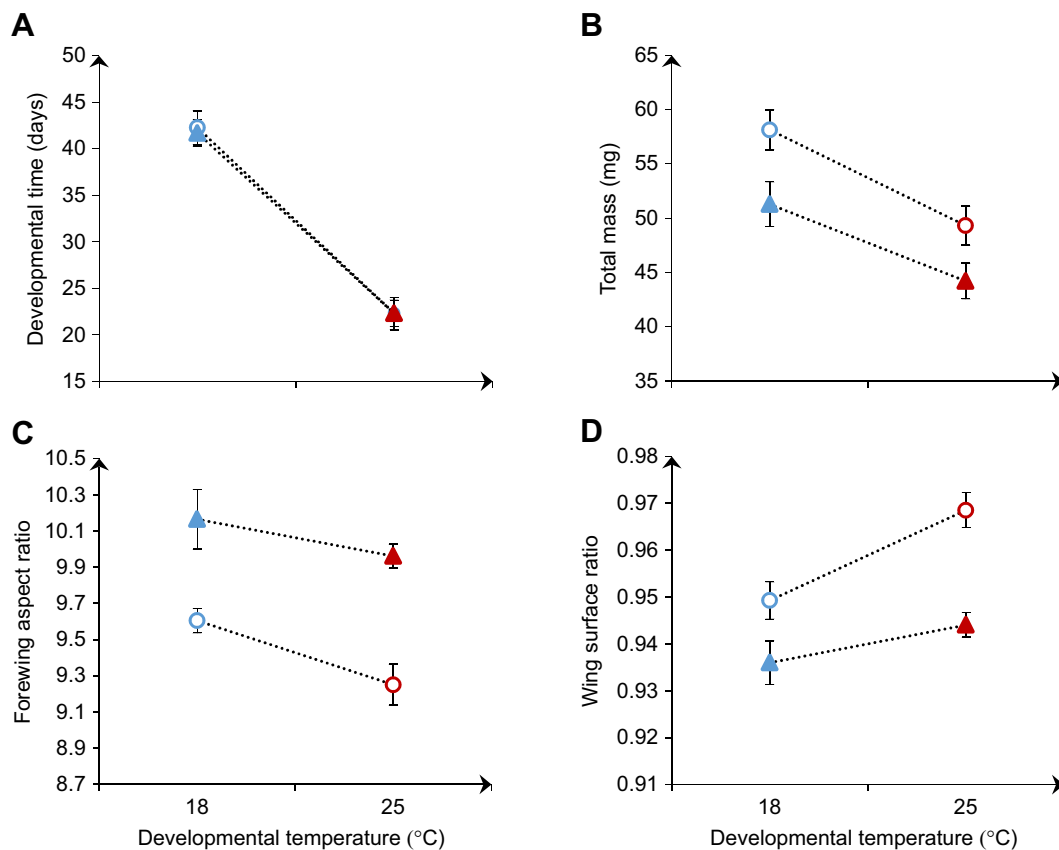


Fig. 3. Impact of developmental conditions on adult morphology. Mean±s.e.m. (A) development time, (B) total adult mass, (C) forewing aspect ratio and (D) wing surface ratio in relation to developmental temperature and sex. Developmental temperature is represented by colour (blue: 18°C, red: 25°C) and sex by shape (circles: females, triangles: males).

Flight performance

Butterflies raised under warm conditions flew further in the tunnel test than those raised under cool conditions (with males flying further than females; Table 1; Fig. 2C). Flight endurance was significantly affected by the interaction between developmental temperature and sex, indicating that females flew longer when raised under cool conditions ($t_{149,40}=2.27$, $P=0.01$) while no significant differences were found between developmental temperatures for males ($t_{148,40}=-0.6$, $P=0.92$, Table 1; Fig. 2D). Endurance (movement capacity) and flight distance (movement propensity) were negatively correlated for females raised under cool conditions ($r_s=-0.35$, $P=0.039$), but positively correlated for males raised under warm conditions ($r_s=0.36$, $P=0.023$).

Internal individual state: fecundity and physiological parameters

Females raised under warm conditions laid only half as many eggs as those raised under cool conditions (Table 1, Fig. 2B). Antioxidant capacity, SOD capacity and DNA damage were significantly higher in individuals raised under warm conditions than in those raised under cool conditions (Table 1, Fig. 4). Moreover, flight metabolic rate was higher in males than in females but was not affected by developmental temperature.

Relationships between morphological parameters and flight performance

In the females raised under cool conditions, flight distance correlated negatively with PC1 ($r_s=-0.40$, $P=0.05$) reflecting measures of body size (mass, wing size and wing loading; Table S3a) and positively with PC3 scores ($r_s=0.41$, $P=0.04$) reflecting wing shape (forewing aspect ratio; Table S3a). No other correlation was found in other developmental groups (all $P>0.6$; Table S2).

DISCUSSION

We expected a change in the pace of life of butterflies depending on the conditions they experienced during their development as a result of an accelerated development under warmer conditions. Our results support this scenario, as warmer developmental conditions were followed by an enhanced flight propensity and self-maintenance but also by a reduced fecundity of *P. napi* butterflies. Importantly, this change in the pace of life of butterflies was observed even though adult butterflies were not experiencing the same thermal conditions as those they previously experienced during their development but

similar intermediate thermal conditions. This suggests that these changes were irreversibly determined during development irrespective of the conditions adults experienced afterwards. Finally, only one negative correlation was found, between body size and flight performance in females developing under cool conditions. The absence of correlations in other groups suggests that other factors, masking the effect of body size on flight performance, are likely to be involved. This shows that the preponderant effects of body size on fitness parameters cannot be easily generalized and depend on developmental conditions.

Self-maintenance versus flight performance

Butterflies raised under warm conditions developed faster and showed higher antioxidant defences than those raised under cool conditions. An accelerated growth, provoked by warm developmental temperature, is typically accompanied by an elevated reactive oxygen species (ROS) production (Smith et al., 2016), and the associated deleterious action on tissues has to be counteracted by antioxidant defences. This suggests that defence mechanisms activated during development persisted later during the pupal stage and at adulthood. Resource allocation into self-defence mechanisms such as antioxidant defences might be traded-off against flight muscle structure and therefore negatively affect flight capacity (Rauhamaäki et al., 2014). Even though the activation of antioxidant defences does not appear complete, as individuals raised under warm conditions still show persistent high levels of oxidative damage at adulthood, this could contribute to the lower flight capacity (i.e. endurance) of females raised under warm conditions. In contrast, the movement capacity of males did not depend on developmental temperature (even though males also showed higher antioxidant defences when raised under warm conditions), possibly because stronger selection pressures on the flight of males (to defend their territory, locate potential mate and practise courtship; Le Roy et al., 2019a) may lead to the canalization of this trait irrespective of developmental stress (Arnaud et al., 2013; Liefting et al., 2009). Accordingly, previous studies found that male insects could maintain performance traits despite variation in physiological (flight performance; Lorenz et al., 2020) or morphological (flight performance; Karl et al., 2008) traits. In contrast to movement capacity, movement propensity (i.e. flight distance) was increased in all individuals (males and females) experiencing warm developmental conditions, possibly reflecting a higher propensity to explore unknown environments (Legrand et al., 2016). In this case, the imbalanced oxidative status observed in these individuals might reflect stressful environmental conditions and

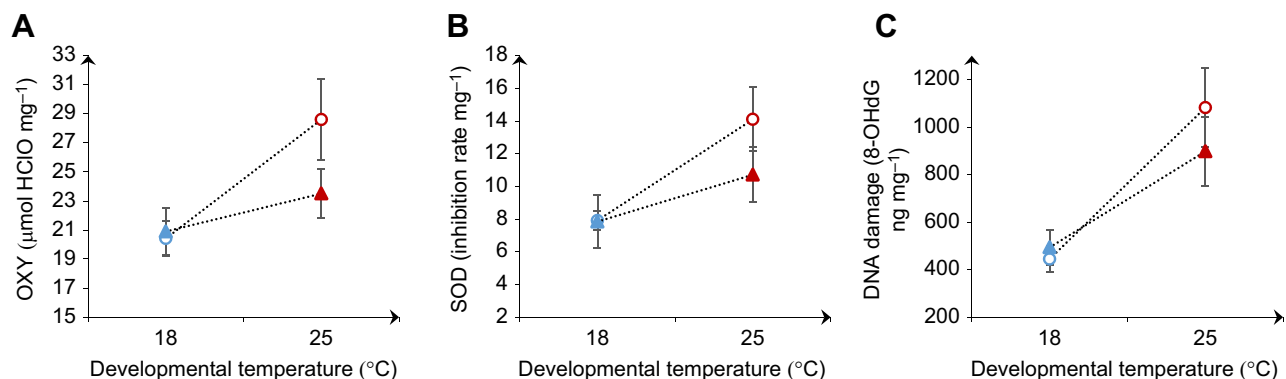


Fig. 4. Impact of developmental conditions on physiological parameters. Mean \pm s.e.m. (A) total antioxidant capacity (OXY), (B) superoxide dismutase (SOD), (C) DNA damage (8-OHdG) in relation to developmental temperature and sex. Developmental temperature is represented by colour (blue: 18°C, red: 25°C) and sex by shape (circles: females, triangles: males).

trigger movement decisions to avoid unfavourable environments (Nowicki et al., 2014).

Reproduction versus self-maintenance

In butterflies, investment into self-maintenance can be traded-off against investment in spermatophore (Kaitala and Wiklund, 1994; Meslin et al., 2018) or egg production (Alonso-Alvarez et al., 2017) if resources are limited. In our study, even though the lower fecundity of females raised under warm conditions may be related to the direct effect of development temperature on their regulation between self-maintenance and reproduction, this lower fecundity may also be the result of the lower quality of the spermatophore transferred by males to females during mating, as high temperatures can also negatively affect sperm quality. However, temperature differences may be sufficient to affect female fecundity, as exemplified by female *Bicyclus anynana* butterflies experiencing hot conditions at adulthood and having reduced fecundity (at least in the first 10 days after mating; Beaulieu et al., 2015). However, these female butterflies also showed higher antioxidant defences and lived longer (Beaulieu et al., 2015), which may ultimately increase their overall fecundity output in the long term (Haeler et al., 2014). For example, in polyandrous species, longer survival increases the possibility of repeated mating, enhancing life-time fecundity output (geometric mean) but coming at a cost of lower direct fecundity output (arithmetic mean; Karlsson, 1998). Switches in mating strategies (monoandrous, biandrous or polyandrous) occur between seasons (Larsdotter-Mellström and Wiklund, 2015; Larsdotter Mellström et al., 2010) and along a latitudinal gradient (Välimäki et al., 2008) in natural populations of *P. napi*. Because the oxidative status of butterflies appears to be determined during their development in our study, it might act as an internal cue triggering future switches between different investment patterns into reproduction, survival and dispersal at adulthood.

Flight performance versus reproduction

The trade-off between flight and reproduction has mostly been examined between flight muscle and ovarian tissue in wing-dimorphic insects, where flight-capable individuals participate mainly in dispersal and less mobile individuals have a greater reproductive output (Mole and Zera, 1993; Zera and Denno, 1997). However, this trade-off in insect species where all adults are supposed to be able to fly and where variation in wing size is small and continuous (e.g. butterflies) may have been overestimated and remains unclear (Tigreros and Davidowitz, 2019). In the context of range expansion, it may be adaptive for dispersive individuals to display both enhanced flight performance and enhanced reproductive output to improve the probability of successful settlement (Hanski et al., 2006; Saastamoinen, 2007). The results of our study (lower fecundity and higher movement propensity in butterflies raised under warm conditions) resemble differences in life-history patterns between the spring and the summer generations in *P. napi*, with individuals from the summer generation (raised under warmer conditions) leaning toward more dispersive life-history traits (Karlsson and Johansson, 2008). Depending on seasonal characteristics and habitat fragmentation, the availability of host plants may change and more dispersive individuals may be at an advantage when host plants are rare. As a result, differences in life-history traits between generations might generate seasonal polyphenism following specific patterns (Karlsson and Van Dyck, 2005; Larsdotter Mellström et al., 2010), with individuals raised under cool conditions having a fitness advantage by investing in early reproduction when food and host plants are abundant, whereas

individuals raised under warm conditions gain a fitness advantage by investing more strongly in dispersal.

Phenotypic plasticity in the context of climate change

Our study provides an insight into the impact of plasticity triggered by developmental conditions, which, through life-history patterns, can increase the probability of population persistence under challenging warmer conditions, at a local scale and without any genetic change (Forsman, 2014; West-Eberhard, 2005). However, because of global environmental changes, unpredictable environmental conditions are expected to be more and more common in the future (Beldade et al., 2011; Moczek et al., 2011). This unpredictability might be problematic for seasonal animals relying on environmental cues encountered during development to adjust life-history strategies at adulthood (Danks, 2007; Singh et al., 2020), as it may increase the probability for adults to experience a mismatch between their phenotype and the environmental conditions they encounter (if the conditions experienced during development deviate from those subsequently experienced by adults). Moreover, more studies are needed to understand the full complexity of the interactions under natural conditions reflecting acclimatization to several factors acting simultaneously on the adult phenotype (Collier et al., 2019). For example, the impact of temperature on individuals but also on their environment such as food and/or host plant availability, might co-influence phenotypic responses (Kuczyk et al., 2021).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.C.D., P.M., M.B., K.F.; Methodology: A.C.D., M.Q., F.C., K.F., P.M., M.B.; Software: A.C.D.; Validation: M.Q., F.C., K.F., P.M., M.B.; Formal analysis: A.C.D., M.B.; Investigation: A.C.D., P.M., M.B.; Resources: M.Q., F.C., K.F., P.M.; Data curation: A.C.D.; Writing - original draft: A.C.D.; Writing - review & editing: A.C.D., M.Q., F.C., K.F., P.M., M.B.; Visualization: A.C.D., P.M., M.B.; Supervision: P.M., M.B., K.F.; Project administration: P.M.; Funding acquisition: K.F., M.B., P.M.

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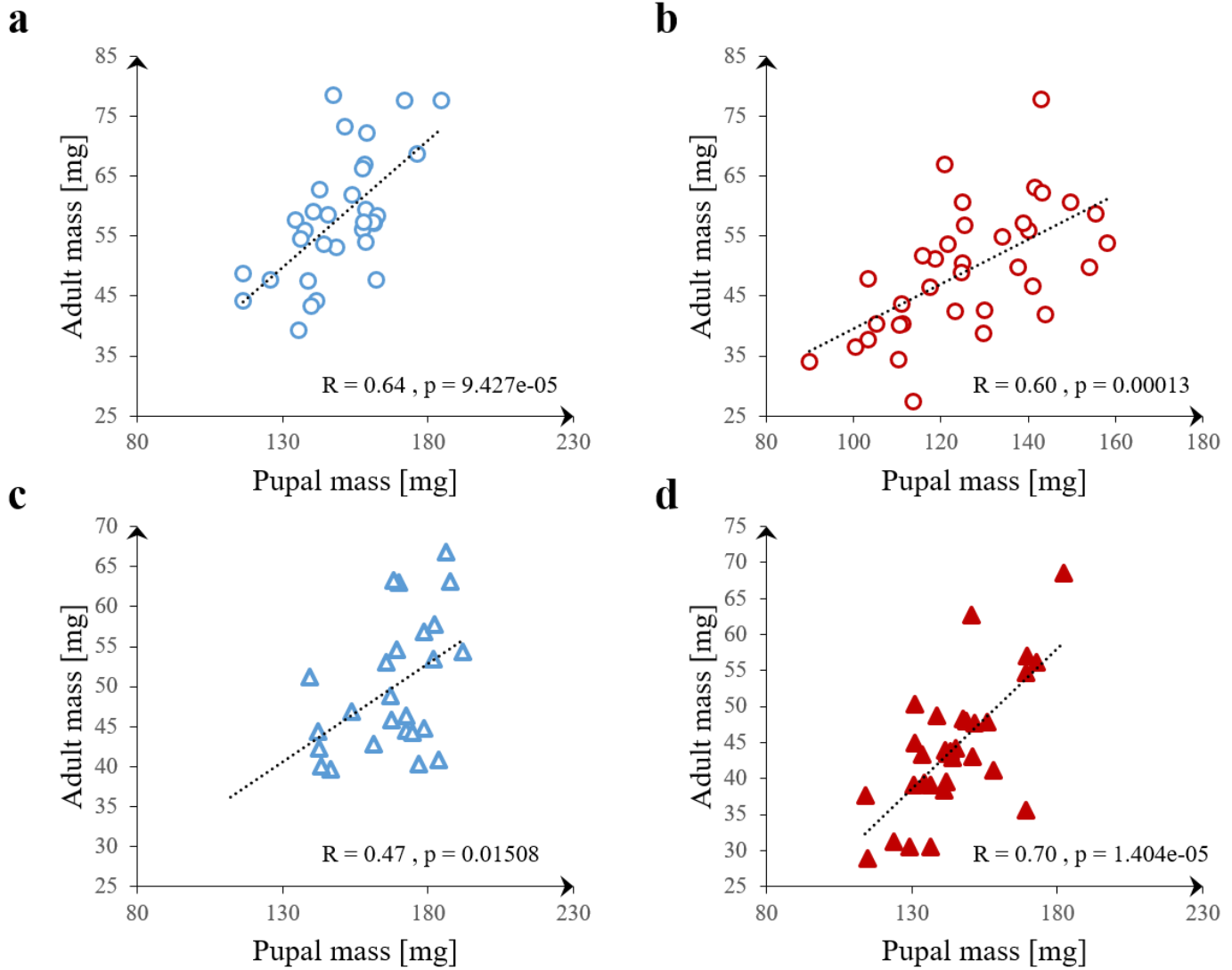


Fig. S1. Linear regressions between pupal mass and adult mass for (a) females developed at 18°C, (b) females developed at 25°C, (c) males developed at 18°C, (d) males developed at 25°C. Developmental temperatures are represented by symbol (circle: 18°C, triangle: 25°C) and sexes by colour (open: females, filled: males).

Table S1. Mean values and standard deviations (SDs) of each trait for males and females at the two developmental temperatures. Different superscript letters within rows show significant differences among treatments (Tukey HSD).

Trait	Females		Males	
	18	25	18	25
	N = 35	N = 45	N = 34	N = 43
Developmental process				
Development time (days)	42.26±1.82 ^(a)	22.30±1.74 ^(b)	41.68±1.43 ^(a)	22.30±1.44 ^(b)
Larval time (days)	28.83±1.62 ^(a)	15.50±1.44 ^(b)	28.65±1.41 ^(a)	15.64±1.36 ^(b)
Pupal time (days)	13.43±0.85 ^(a)	6.80±1.02 ^(b)	13.03±0.58 ^(a)	6.71±0.50 ^(b)
Pupal mass (mg)	14.89±1.66 ^(a)	12.71±1.63 ^(b)	16.23±1.95 ^(c)	14.37±1.56 ^(d)
Weight loss	61.16±5.38 ^(a)	60.93±6.63 ^(a)	69.35±6.15 ^(b)	69.5±4.53 ^(b)
Adult mass				
Total mass (mg)	58.14±10.41 ^(a)	49.32±10.64 ^(b)	51.28±10.47 ^(a)	44.21±09.13 ^(c)
Thorax mass (mg)	18.82±2.53 ^(a)	15.3±3.03 ^(b)	20.73±2.69 ^(a)	17.740±3.39 ^(c)
Abdomen mass (mg)	27.72±8.08 ^(a)	24.3±8.08 ^(a)	17.87±8.96 ^(b)	15.31±5.21 ^(b)
Relative thorax mass	0.72±0.18 ^(a)	0.68±0.22 ^(a)	1.32±0.38 ^(b)	1.23±0.27 ^(b)

Wing morphology

Wing area (cm ²)	4.37±0.38 ^(a)	3.86±0.49 ^(b)	4.81±0.36 ^(c)	4.24±0.44 ^(d)
Forewing length (cm)	2.26±0.09 ^(a)	2.09±0.16 ^(b)	2.43±0.14 ^(c)	2.26±0.10 ^(d)
Aspect ratio Forewing	9.60±0.34 ^(a)	9.25±0.66 ^(a)	10.16±0.76 ^(b)	9.96±0.36 ^(b)
Wing surface ratio	0.95±0.02 ^(a)	0.97±0.02 ^(b)	0.94±0.02 ^(a)	0.94±0.01 ^(c)
Wing loading	13.33±1.60 ^(a)	13.03±2.36 ^(a)	10.41±1.53 ^(b)	10.38±1.36 ^(b)

Mobility

Endurance (sec)	248.03±170.8 ^(a)	169.60±165.92 ^(b)	197.67±159.06 ^(b)	227.23±188.47 ^(b)
Distance (score)	4.2±1.45 ^(a)	6.46±2.15 ^(b)	7.91±2.17 ^(c)	9.93±2.08 ^(d)

Physiology

Flight metabolic rate (ppm/min)	27.11±9.89 ^(a)	26.84±10.76 ^(a)	33.23±11.49 ^(b)	29.04±10.59 ^(b)
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Fecundity

Number of eggs	32.97±5.75 ^(a)	16.5±2.86 ^(b)
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Oxidative status

Total antioxidant capacity (OXY)	20.41±4.94 ^(a)	28.60±10.53 ^(b)	20.90±6.91 ^(a)	23.50±6.55 ^(a)
Superoxide dismutase (SOD)	7.91±2.38 ^(a)	14.09±7.31 ^(b)	7.84±3.42 ^(a)	10.71±4.30 ^(b)
8-OHdG (DNA damage)	444.29±227.04 ^(a)	1081.00±625.4 ^(b)	493.23±318.25 ^(a)	896.96±559.82 ^(b)

Table S2. Results of general linear models with PCs 1-4 coordinates for each developmental group. Significant *P*-values are shown in bold.

Trait	Females		Males	
	18	25	18	25
Distance				
PC1	$F_{1,43}=4.99$, P=0.04	$F_{1,0.1}=0.02$, P=0.88	$F_{1,0.1}=0.01$, P=0.96	$F_{1,21}=3.6$, P=0.07•
PC2	$F_{1,0.1}=0.02$, P=0.89	$F_{1,1}=0.20$, P=0.66	$F_{1,1.4}=0.01$, P=0.76	$F_{1,0.1}=0.01$, P=0.98
PC3	$F_{1,44}=5.15$, P=0.03	$F_{1,0.1}=0.01$, P=0.96	$F_{1,0.1}=0.01$, P=0.97	$F_{1,8}=1.41$, P=0.25
PC4	$F_{1,4}=0.50$, P=0.49	$F_{1,0.7}=0.12$, P=0.73	$F_{1,4}=0.29$, P=0.59	$F_{1,5}=0.92$, P=0.35
Endurance				
PC1	$F_{1,44624}=1.30$, P=0.27	$F_{1,4041}=0.15$, P=0.71	$F_{1,59}=0.01$, P=0.96	$F_{1,1847}=0.05$, P=0.82
PC2	$F_{1,4121}=0.12$, P=0.73	$F_{1,1478}=0.05$, P=0.82	$F_{1,100601}=3.39$, P=0.08•	$F_{1,1036}=0.03$, P=0.87
PC3	$F_{1,1408}=0.04$, P=0.84	$F_{1,22915}=0.83$, P=0.37	$F_{1,4627}=0.16$, P=0.69	$F_{1,12289}=0.34$, P=0.57
PC4	$F_{1,45136}=1.31$, P=0.27	$F_{1,60350}=2.18$, P=0.15	$F_{1,72349}=2.44$, P=0.14	$F_{1,58966}=1.62$, P=0.22

Table S3. Results of the principal component analyses for females at (a) 18°C and at (b) 25°C and for males at (c) 18°C and at (d) 25°C with Eigen values, proportion of variance explained, cumulative proportions of variance explained, and r-values of the correlations between morphological variables and principal components. Significant correlations $P < 0.05$ in bold.

Table S3a

	PC1	PC2	PC3	PC4
Eigen value	3.430	2.010	1.157	0.993
Variance explained (%)	42.9	25.1	14.5	12.4
Cumulative variance (%)	42.9	68	82.5	94.9
Thorax mass	0.767	0.459	-0.251	0.249
Abdomen mass	0.899	-0.412	-0.097	0.026
Relative thorax mass	-0.483	0.807	-0.064	0.165
Mean wing area	0.807	0.522	0.220	-0.030
Forewing length	0.742	0.340	0.542	0.169
Forewing aspect ratio	-0.136	-0.531	0.754	0.206
Wing surface ratio	-0.371	-0.076	-0.102	0.893
Wing loading	0.666	-0.550	-0.399	0.186

Table S3b

	PC1	PC2	PC3	PC4
Eigen value	2.845	2.430	1.224	0.967
Variance explained (%)	35.6	30.4	15.3	12.1
Cumulative variance (%)	35.6	65.9	81.2	93.3
Thorax mass	0.732	0.275	0.569	0.096
Abdomen mass	0.770	-0.595	0.093	-0.138
Relative thorax mass	-0.323	0.784	0.356	0.195
Mean wing area	0.689	0.639	0.082	-0.149
Forewing length	0.789	0.521	-0.223	0.179
Forewing aspect ratio	0.413	-0.01	-0.688	0.545
Wing surface ratio	0.538	0.784	0.356	0.893
Wing loading	0.690	0.640	0.082	0.186

Table S3c

	PC1	PC2	PC3	PC4
Eigen value	3.022	2.177	1.274	0.988
Variance explained (%)	37.8	27.2	15.9	12.4
Cumulative variance (%)	37.8	65	80.9	93.3
Thorax mass	0.784	0.075	-0.476	0.476
Abdomen mass	0.939	-0.293	-0.002	-0.098
Relative thorax mass	-0.655	0.362	-0.159	0.596
Mean wing area	0.366	0.603	-0.554	-0.403
Forewing length	0.447	0.886	-0.030	-0.087
Forewing aspect ratio	0.295	0.708	0.409	0.324
Wing surface ratio	0.076	0.274	0.825	-0.223
Wing loading	0.818	-0.480	0.163	0.266

Table S3d

	PC1	PC2	PC3	PC4
Eigen value	4.312	1.452	1.240	0.519
Variance explained (%)	53.9	18.1	15.5	6.492
Cumulative variance (%)	53.9	72	87.5	94
Thorax mass	0.912	0.266	-0.297	0.297
Abdomen mass	0.915	-0.360	0.113	0.078
Relative thorax mass	-0.215	0.834	-0.410	0.286
Mean wing area	0.854	0.380	0.294	-0.199
Forewing length	0.687	0.389	0.582	-0.108
Forewing aspect ratio	-0.663	-0.187	0.485	0.282
Wing surface ratio	-0.559	0.170	0.613	0.269
Wing loading	0.803	-0.442	-0.122	0.374

Table S4. Full citation of R packages used for the statistical analyses

Title	Author	Year	Note	URL
survival: A Package for Survival Analysis in R	Terry M Therneau	2021	R package version 3.2-13	https://CRAN.R-project.org/package=survival
lme4: Linear mixed-effects models using Eigen and S4	Douglas Bates and Martin Maechler and Ben Bolker and Steven Walker	2015	R package version 1.1-8	http://CRAN.R-project.org/package=lme4
emmeans: Estimated Marginal Means, aka Least-Squares Means	Russell V. Lenth and Paul Buerkner and Maxime Herve and Jonathon Love and Hannes Riebl and Henrik Singmann	2021	R package version 1.7.0	https://CRAN.R-project.org/package=emmeans
factoextra: Extract and Visualize the Results of Multivariate Data Analyses	Alboukadel Kassambara and Fabian Mundt	2020	R package version 1.0.7.	https://CRAN.R-project.org/package=factoextra