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Adaptation of a tropical butterfly to a temperate climate

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GENERAL INFORMATION

Title: Adaptation of a tropical butterfly to a temperate climate

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ABSTRACT

Developmental plasticity enables organisms to cope with environmental heterogeneity, such as seasonal variation in climatic conditions, and is thought to affect a species' capability to adapt to environments with novel seasonal and ecological dynamics. We studied developmental plasticity of the widespread tropical butterfly, *Bicyclus safitza*, which reaches the southern edge of its distribution in the temperate zone of South Africa. In wet-dry seasonal environments in tropical Africa, adults of *Bicyclus* butterflies are present all-year-around and exhibit discrete seasonal forms in alternating generations. We demonstrate that a population that colonized a more temperate climate region has adopted a different strategy to cope with local environment as no active adults were encountered during the temperate winter. The flight season coincided with a period when evaporation stress was lowest and temperatures were higher in the South African population. Butterflies collected from the field did not express seasonal polyphenism or show full expression of the tropical wet season form phenotype. Reaction norm experiments comparing stocks from South Africa and Uganda indicated that local adaptation of this tropical butterfly to a more temperate climate involved the evolution of developmental plasticity, such that a more robust development in response to thermal variation was observed for a broad suite of morphological and life history traits. Our findings have implications for understanding the mechanisms that facilitate expanding into a novel ecological niche under seasonally variable climatic conditions.

INTRODUCTION

Natural environments are heterogeneous and many insect species have evolved strategies to cope with spatial and temporal environmental variation. Typically these involve dispersal from unfavourable to favourable conditions, either in space by means of migration, or in time by inducing a state of arrested development or diapause (Bohonak and Jenkins 2003). Compelling examples of both phenomena include the annual migrations of monarch butterflies *(Danaus plexippus)* and the winter diapause in many other temperate butterflies, such as the speckled wood butterfly *(Pararge aegeria)*, which is induced when changes in day length indicate that winter is coming (Aalberg Haugen and Gotthard 2015). An alternative strategy to cope with environmental variability is seasonal polyphenism; an extreme example of developmental plasticity in which different forms of a species are produced at different times of the year. Seasonal forms have adaptive traits specific to the environment in which they occur (Shapiro 1976). In contrast to spatially or temporally dispersing insects in the tropics, seasonally polyphenic species remain active throughout the year and, thus, demonstrate less dramatic seasonal peaks in activity and abundance.

Bicyclus butterflies have become a hallmark example of adaptive developmental plasticity and seasonal polyphenism as they have proved a tractable system in which to study the environmental regulation of development in both natural (Brakefield and Reitsma 1991, Windig et al. 1994) and laboratory populations (de Jong et al. 2010, Oostra et al. 2014, van Bergen et al. 2017). The genus consists of over 100 extant species that inhabit a wide range of tropical habitats in sub-Saharan Africa (Aduse-Poku et al. 2015). Recent work has supported that a single *Bicyclus* species began to colonize more open and seasonal habitats during the Miocene epoch, when much of the trans-continental forests began to open up (Aduse-Poku et al. 2015). Such environments, with highly distinct wet and dry seasons, are characterized by predictable patterns of variation in temperature, rainfall and humidity that

are closely associated with changes in vegetation cover and host plant availability (e.g. Brakefield and Larsen 1984). The rains of the wet season result in a luxuriant growth of herbs, including species of grass, which the larvae of most *Bicyclus* species use as host plants. Adult butterflies in the middle of the warm, wet season are highly active and reproduce quickly (Brakefield and Reitsma 1991). Larvae of this generation develop in increasingly arid and cooler conditions towards the dry season when the ground vegetation, including larval host plants, dies back to become a layer of brown leaf litter. The next generation of adult butterflies emerges around the transition between wet and dry season, and then survives the unfavourable conditions as active, but reproductively dormant, adults before reproducing at the beginning of the next wet season (Brakefield and Reitsma 1991, Windig et al. 1994, van Bergen et al. 2016).

In the laboratory, development of phenotypes similar to the seasonal forms found in nature can be induced by manipulating the temperature during a sensitive phase of pre-adult development (Kooi and Brakefield 1999). Larvae reared at low temperatures, which represent the environmental conditions of the tropical dry season, develop into relatively large individuals, which allocate resources toward a more durable body and demonstrate cryptic patterning of the ventral wings that are exposed when at rest. In contrast, wet season form individuals, which are induced by high developmental temperatures, have a series of conspicuous marginal eyespots on their ventral wing surfaces, and demonstrate an increased investment in reproduction. The distinct wing patterns serve an important fitness function in terms of coping with changing predatory threats between seasonal environments (Lyytinen et al. 2004, Prudic et al. 2015).

The common bush brown, *Bicyclus safitza* (Westwood, 1850), is one of the most widely distributed species of *Bicyclus* butterflies (e.g. Larsen 2005). Whereas all other species of *Bicyclus* are restricted to tropical climate zones, the distribution of *B. safitza*

extends far into more temperate climate zones in southern Africa. One of the most fundamental differences between temperate and tropical ecosystems is the ambient temperature during winter, which can even drop below freezing in temperate regions, and populations of tropical butterflies which successfully colonized more temperate climate regions are predicted to have evolved a suite of adaptations to cope with local environmental conditions. For example, recent field studies have revealed that populations of *B. safitza* in these temperate regions have a strong preference for shaded forests habitats (Nokelainen et al. 2016), which may buffer seasonal fluctuations in temperature and humidity, whereas in tropical biomes *B. safitza* is mainly found in semi-open woodland and forest edge habitats (Brakefield and Reitsma 1991, Windig et al. 1994).

Here, we explore whether a population of *Bicyclus safitza* that occurs in Eastern Cape of South Africa has become locally adapted to temperate climatic conditions. We also aim to find explanations for the strong population-specific preference for more shaded habitats (Nokelainen et al. 2016). To address these questions, we study the phenology and the expression of seasonal polyphenism of temperate population by conducting a longitudinal survey in the Eastern Cape, South Africa, and compare these to a well-studied tropical population of *B. safitza* (Brakefield and Reitsma 1991, Windig et al. 1994). Secondly, using the ratios of stable isotopes of oxygen obtained from the exoskeleton of field-trapped individuals as well as measurements of local water evaporation during the larval stage, we explore whether evaporation stress may constrain the butterfly niche. Finally, we conducted a comparative reaction norm study, using populations from temperate and tropical climate regions, to investigate the extent of local adaptation in response to different thermal environments. Using data from these two populations, we aim to provide insights on the mechanisms that facilitate the colonization of novel ecological niches under seasonally variable climatic conditions.

MATERIALS AND METHODS

Field sites and butterfly monitoring

We monitored butterflies at the southern-most southern edge of the species' range in the Eastern Cape province of South Africa. The Eastern Cape spans over a multitude of climatic regions including cold and temperate interior parts as well as temperate and sub-tropical coastal regions (Mucina and Rutherford 2006). Our study sites were predominantly in a temperate zone that is characterised by open, semi-arid grasslands, whereas afromontane forests and coastal thickets provide more humid, shaded-habitats. The field sites in the vicinity of Grahamstown and more humid coastal and riverine environments were chosen using satellite images (Google Earth, Google Inc., Mountain View, CA, USA) to detect suitable habitats after which the areas were visited to confirm the presence of *B. safitza*. Three field sites were used in this study: Bathurst (33°30'S, 26°46'E), Kapriver (33°21'S, 26°52'E) and Kasouga (33°39'S, 26° 44'E). The Bathurst site represents a riparian bush habitat with more open areas along the edges. The Kapriver site is an open, grassy hilltop, which transitions into riparian forest in a lower lying river gorge. The Kasouga site is characterised by coastal thickets and bordered by pastureland to the north, and the shores of the Indian Ocean to the south.

To investigate the seasonal phenology of *B. safitza* in temperate regions, we monitored field populations by conducting monthly trapping sessions between November 2014 and October 2015. Nine traps (Megaview, DC0017, Pop-up Butterfly Bait Trap, cone type) were placed at each of the three field sites and equally distributed among three habitat types, open grasslands, forest fringes and under shaded canopy, within each site (for further details and habitat preference comparisons, see Nokelainen et al. 2016). These traps were baited with fermented banana once a month and emptied on the following day. Wild-caught

individuals were stored in entomological envelopes until further processing. Measurements of relative humidity and temperature were obtained from each of the three habitats at all three sites using data loggers (Maxim DS1922T iButton Temperature Logger, San Jose, CA, USA) to enable comparisons of climatic conditions. Data on the phenology and wing pattern plasticity of tropical populations have been published by Brakefield and Reitsma (1991) and Windig *et al.* (1994), and here we use the same methodologies to quantify habitat use and variation in butterfly occurrence and wing patterning.

Evaporation stress measures

Evaporation stress from wild-caught butterflies was first studied using the ratio of stable isotopes of oxygen ($\delta^{18}O$) present in the exoskeleton, which have been shown to reflect the mean atmospheric conditions surrounding the insect before moulting (Ellwood et al. 2011). Briefly, the more common, lighter ^{16}O-isotope evaporates more readily than the ^{18}O-isotope, which leads to enrichment of ^{18}O in sample tissues and thus, more positive $\delta^{18}O$-values. To quantify the $\delta^{18}O$-values of the specimens collected at the field sites, two legs were placed into silver capsules, sealed and loaded into an auto-sampler. The tissue within the capsule was pyrolysed at 1200^{o}C using a Thermo Finnigan TC/EA attached to a Thermo Delta V mass spectrometer via a ConFlo 3. Reference standards from IAEA in Vienna were run at intervals throughout the sequence and these values are used to calibrate to the international standards of $^{18}O/^{16}O$ ($\delta^{18}O$ V-SMOW). Analyses were conducted at the Godwin Laboratory for Palaeoclimate Research, Department of Earth Sciences at University of Cambridge, UK.

We measured larval rates of water loss with Li-Cor 6400 photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Three separate runs were conducted using a total of nine similar-sized 3rd instar larvae of *B. safitza* ($\bar{x}$ = 22.5 mg). Larvae originated from an F1-laboratory stock, initially collected from the Kasouga field site, and were used only once. For

the measurements, three larvae were placed together (for better measurement accuracy) in a small mesh cage that was inserted into the leaf chamber of the photosynthesis system in order to obtain rates of water-loss. The device measures the exchange of CO_2 and H_2O between organism and atmosphere, controlling ambient CO_2 concentration, temperature and relative humidity and hence, the vapour pressure deficit (VPD, the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated). The conditions under which an organism maintains its water balance during temperature changes are more clearly shown by noting the VPD than the relative humidity (Anderson 1936). Based on this, we measured larval evaporation rates in response to five different VPD conditions (VPD [kPa] = 1.5, 2, 2.5, 3, 3.5) at a constant ambient temperature of 25°C. Evaporation measures were started at the lowest VPD and successively increased. Once a target VPD was attained, five water loss measures (over ten seconds duration) were recorded and averaged. Values were used to calculate average water loss as a percentage of larval body mass per hour (% H_2O g^{-1} hr^{-1}). Climate data at the study sites was then used to calculate natural range of VPD's (Table 1) and predicted larval water loss based on the relationship established in the laboratory (see supplementary information).

Temperature reaction norm experiment

To study the geographic variation in the degree of developmental plasticity we conducted a reaction norm experiment using two populations of *B. safitza* and four constant thermal regimes. The laboratory populations of *B. safitza* were established in 2013 from eggs collected at a single location in the Semuliki National Park in Uganda (0°50'N, 30°9'E) and the Kasouga field site in South Africa (33°39'S, 26°44'E). The eggs from at least ten females contributed to each stock population. Thus, the Ugandan colony was derived from a population in the tropics whereas the South African colony originated from a temperate

population at the poleward margin of the species range. After about four generations of laboratory rearing, eggs were collected from both laboratory stocks and larvae were randomly divided over four climate-controlled chambers (21°C, 23°C, 25°C and 27°C) within one day after hatching. In these chambers (Sanyo/Panasonic MLR-350H, 70% RH, 12:12 L:D cycle), larvae were reared in sleeve-like gauze cages on young wheat (*Triticum aestivum*) plants: a host plant that is frequently used to rear newly established laboratory populations of *Bicyclus* butterflies (Oostra 2014, van Bergen 2017). Pre-pupae were collected daily and one day after pupation they were weighed to the nearest 0.1 mg (Fisherbrand PS-60) and individually placed in transparent pots until they eclosed. On the first day after eclosion the adults were sacrificed by freezing and carefully dissected. In addition to wing pattern measurements (see below) we recorded the larval and pupal development times, calculated the growth rate, and measured adult dry mass, relative fat content and abdomen ratio. Data from the Ugandan population were included in a comparative study on developmental plasticity in mycalesine butterflies, for details on methodology see van Bergen *et al.* (2017).

Wing pattern measurements

The ventral surface of one hind- and one forewing of each individual were photographed using a Leica DFC495 digital camera with a Leica M125 stereomicroscope. The images were analysed with the image processing package Fiji (Schindelin et al. 2012). We followed the Comstock-Needham system to refer to wing veins and cells (see Miller 1970). On the ventral hind wing, the area of the yellow outer ring, the black inner-disc, and white focus of the eyespot in cell Cu1 were measured. The relative distance of the proximal edge of the median band along the second wing vein was taken as a measure of the width of the band. The measurements on the ventral forewing included the yellow, black and white areas of the eyespot in cell M1 as well as area of the black inner-disc of the larger eyespot in cell Cu1.

For all wings an area enclosed by three clear landmarks was used as a proxy of wing size (Figure 1). The same protocol was used for both experimental and field-caught individuals.

Statistical analyses

Prior to analyses, all wing pattern elements were corrected for wing size and the nine ventral wing-pattern measurements (traits 1-5; see Fig. 1) were reduced using a principal component analysis, pooling all available data. The first principal component (PC1) explained 60 per cent of the total variation and was strongly associated with the effect of the developmental temperature and month of capture. PC2 explained 15 per cent of the variation and was correlated with sex rather than seasonality. In addition, all development times from temperature reaction norm experiment were log-transformed to improve normality. Statistical analyses were performed with the R Statistical Package v 3.1.2 (R Development Core Team 2014) and IBM SPSS Statistics (v22). For the field-collected data, 3-way ANOVAs were used to analyse the effect of monthly mean temperature, sex and sampling site on wing pattern morphology (PC1) and butterfly dry mass. Full models were fitted including temperature, sex, population and their interactions, before successive removal of non-significant terms. To investigate seasonal phenology of the butterflies, we tested expected equal monthly occurrence of butterflies (i.e. frequency of captured butterflies) across months using Chi-Square tests.

We used 3-way ANOVAs to investigate evaporation stress. For stable oxygen isotope data collected from adults, we used the $\delta^{18}O$ values as the dependent variable and monthly mean temperature, sex and sampling population and their interactions as explanatory variables. In addition, 3-way ANOVAs were used to analyse the effect of monthly mean temperature, population and habitat on predicted larval water loss (i.e. proportional loss of body mass per hour).

For the reaction norm experiment, 3-way ANOVAs were used to analyse the effect of developmental temperature, sex and population on each phenotypic trait of interest. Full models were fitted initially before successive removal of non-significant terms. The degree of plasticity was estimated by calculating the effect size (Hedges's g), using the means and standard deviation of the data from 21°C and 27°C, for each sex and population separately.

RESULTS

Seasonal phenology and plasticity in the wild

Butterfly monitoring revealed that the number of butterflies varied across months (χ^2 = 353.46, df = 8, $p < 0.001$), with a clear absence of adult activity between May and August (Figure 2). Out of 490 *B. safitza* recorded, males were overrepresented with approximately 3:1 ratio in comparison to females.

Variation in ventral wing pattern morphology (PC1) of field-collected specimens of South African *B. safitza* was best explained by the monthly average temperature ($F_{5,161}$ = 12.20, $p < 0.001$) while adult dry mass showed a three-way-interaction: apart from one sampling site, females were heavier than males and female dry mass varied significantly depending on the month of capture, whereas it remained similar in males throughout the survey. However, dry mass was also dependent of the sampling site and the month of capture varied between sites (3-way-interaction among sex, month and site, $F_{4,202} = 9.87$, $p < 0.001$).

Evaporation stress

Stable isotopes of oxygen ($\delta^{18}O$) indicated that month ($F_{5,227}$ = 26.24, $p < 0.001$) and site ($F_{2,227}$ = 12.20, $p < 0.001$) both influenced evaporation stress as measured from adult butterflies. However, there was also an interaction between the month and site ($F_{8,227} = 2.66$, $p < 0.008$), and the main flight season coincided with a period when $\delta^{18}O$-values were lowest (min = 20.03) in contrast to the end of the season with highest $\delta^{18}O$-values (max = 32.84).

This effect, however, was not as strong in the Kapriver population. The lowest δ^{18}O-values were recorded at Kasouga ($\bar{x}$ = 24.84, n = 160, s.d. = 2.57), followed by Bathurst ($\bar{x}$ = 25.24, n = 7, s.d. = 2.28), whereas butterflies from Kapriver mirrored a higher and more variable evaporation stress ($\bar{x}$ = 25.61, n = 76, s.d. = 2.63).

To explore further how evaporation stress may constrain the butterfly niche, we used the laboratory-established physiological relationship to predict larval water loss with respect to abiotic conditions through the monitoring period. We found two interactions that significantly influenced larval water loss: an interaction between site and month ($F_{8,8857}$ = 5.00, $p < 0.001$), and between site and habitat ($F_{3,8857}$ = 14.02, $p < 0.001$). The main effects of site ($F_{2,8857}$ = 143.39, $p < 0.001$), month ($F_{5,8857}$ = 10.98, $p < 0.001$) and habitat also influenced predicted larval water loss ($F_{2,8857}$ = 103.71, $p < 0.001$). Both temperature and humidity fluctuations through the survey period were less dramatic under the shaded-forest canopy than in forest fringes or grasslands (Table 1).

Plastic responses to developmental temperature

In total, 728 individuals were reared in the temperature reaction norm experiment. We found significant interactions between population and temperature for most life history traits and wing pattern elements, indicating that populations respond differently to developmental temperature (see supplementary material for all minimum adequate models). For all traits, except for the pupal development time in females, the degree of plasticity was larger in the Ugandan population (Figure 3) and the phenotypic differences between populations were wider at higher temperatures. Not all traits were equally plastic in their response to developmental temperature. Relative to other wing pattern elements, the plastic response of the large eyespot on the forewing (2), as well as the width of the ventral bands (4-5) was less pronounced in both populations (Figure 3).

The relationships between developmental temperature and phenotypic variation in the South African population were linear or continuous in most traits. In contrast, the Ugandan population showed more discontinuous responses to temperature (Figure 4). For example, the conspicuousness of the wing pattern elements (PC1) increased dramatically between 23°C and 25°C in Ugandan population and, as a consequence, all individuals reared at the extremes of the temperature gradient showed a close phenotypic resemblance. The difference in the shape of the reaction norm was even more pronounced when using developmental time as a proxy for environmental variation (Figure 4).

DISCUSSION

We show that a natural population of *Bicyclus safitza* in temperate South Africa displays a different seasonal phenology and reduced developmental plasticity in comparison to a population from a tropical region in Uganda. In the tropics, populations of *B. safitza* are typically found in wet-dry seasonal habitats and active butterflies are present here throughout the year. They exhibit discrete seasonal forms, one with conspicuous, and one with cryptic wing patterns (Windig 1991, Brakefield and Reitsma 1991, van Bergen et al. 2016). In contrast, our data show that field-collected specimens in South Africa displayed continuous morphological variation throughout the year with no individuals showing the large eyespots typical of the wet season form in the tropical regions. Moreover, a flight season, in which adult butterflies were active, was followed by a period when no active adults were encountered.

Populations of *B. safitza* that inhabit tropical environments typically survive the unfavourable conditions of the dry season as semi-active adults that will feed opportunistically on fruit, and freely and continuously entered fruit-baited traps (Brakefield and Reitsma 1991, Windig et al. 1994). Our results reveal that a population that colonized a

more temperate climate region in southern Africa has adopted a different strategy to cope with local environmental conditions. In South Africatwo large activity peaks in November and February, and a smaller increase in butterfly numbers in April, were followed by a quiescent period in which no butterflies were caught until late September. These data suggest that this tropical butterfly species takes on a different 'overwintering' strategy in temperate climates, possibly with butterflies aestivating as fully dormant individuals in shelters or surviving in an arrested stage of pre-adult development (Stålhandske et al. 2017).

The adjusted phenology as well as the preference for more shaded habitats at the range margin of the species' distribution may be associated with different evaporation constraints. Stable isotopes of oxygen, which in butterflies reflect evaporation rates during the late larval development (van Bergen et al. 2016), indicated that individuals caught at the beginning of the flight season experienced high evaporation rates during development. In contrast, we observed less evaporation stress in the middle of summer months while larval evaporation rates rose again closer towards the end of the flight season. The quiescent period of adults could thus reflect coping with evaporation stress and as well as targeting the more favourable environmental conditions for reproduction (Brakefield and Larsen 1984, Brakefield and Reitsma 1991). Moreover, larval evaporation rates were predicted to be significantly higher in forest fringes and open grassland compared to more shaded habitats. In the shaded forests the temperature was mild, humidity high and vapour pressure deficit low, which provides a buffer against the weather extremes (Addo-Bediako et al. 2001, Chown et al. 2011). Thus, it is possible that the maintenance of the body water balance, together with plant–insect co-evolution (Braschler and Hill 2007, Nokelainen et al. 2016), constrains populations of *B. safitza* to microclimates provided by shade-habitats in temperate zone.

Our results show that local adaptation of this tropical butterfly species to more temperate climatic conditions in southern Africa involved the evolution of developmental

plasticity. Field surveys confirmed the absence of polyphenism in the wild and, when compared in the laboratory to a population from the tropics, the South African population showed more robust development in response to thermal variation for a broad suite of morphological and life history traits. In addition to geographical variation in the degree of plasticity (i.e. the steepness of the reaction norm), we also observed clear differences in the shapes of reaction norms between the two populations. The expression of ventral wing pattern elements responded in a discontinuous manner to the temperature gradient, which is typical of polyphenism, while the relationship between developmental temperature and phenotypic variation in the South African population was more or less linear for these traits.

The evolution of developmental plasticity in this group of butterflies has been studied by conducting artificial selection experiments using *B. anynana*, a species closely related to *B. safitza* and a model system in the field of eco-evo-devo (Brakefield et al. 2009). Rapid responses to selection were observed with respect to the height of reaction norms (intercept), while developmental plasticity for eyespot size was retained in the selection lines (Brakefield et al. 1996). In contrast, attempts to change the slope (steeper or shallower) or the shape of the reaction norms were largely unsuccessful (Wijngaarden et al. 2002), which suggested that the slope of reaction norms is unlikely to evolve as readily as the intercept. Subsequent studies using two different tropical populations of *B. anynana* from different latitudes revealed parallel reaction norms for a suite of traits and no obvious genotype-by-environment interactions (de Jong et al. 2010), confirming the results obtained in the laboratory. Moreover, recent work confirms that intra-population genetic variation for plasticity is highly depleted in *B. anynana* (Oostra et al. 2017), which may hinder expansions of this species into environments with different seasonal and ecological dynamics. The striking differences in plasticity among populations described in the present study indicate that natural populations of *B. safitza* contain sufficient genetic variation in the response to thermal variation for

developmental plasticity to evolve. In insects, including *Bicyclus anynana* (Koch et al. 1996, Mateus et al. 2014, Monteiro et al. 2015), developmental plasticity is often mediated by endocrine signalling (Nijhout 1999, Zera et al. 2007). The evolution of environmentally sensitive traits, as shown here for *B. safitza*, likely involved evolutionary changes in the levels and timing of systemic hormone titres in response to external cues or in the degree and timing of the sensitivity of hormonal receptors in the developing target tissues.

Finally, phenotypic plasticity not only enables organisms to cope with environmental heterogeneity, such as seasonal variation in climatic conditions, but it may also enable dispersal into regions with climates to which organisms are not adapted to at source (West-Eberhard 2003, Wund et al. 2008, Gibert 2017). Upon exposure to novel environmental variation, plasticity provides an immediate shift in phenotypic variation, leading to increased population persistence and providing time for adaptive evolution to take place. Based on the data from the two populations studied here, we postulate that developmental plasticity of the ancestral population of *B. safitza* may have facilitated the process of local adaptation to temperate climates. Interestingly, individuals from the extant populations mate and produce viable offspring in the laboratory (personal observations). However, hybrid females demonstrated signs of reduced fertility, which is in line with Haldane's rule: the preferential hybrid sterility of the heterogametic sex. This may imply that the South African population of *B. safitza* is becoming genetically isolated and may be on its way to evolving full reproductive isolation.

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manuscript. All authors read and approved the final version of the manuscript for publication.

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FIGURE LEGENDS

Figure 1: Wing pattern elements measured in all specimens of *Bicyclus safitza*. The images represent the typical phenotype of individuals reared at 27°C (row 1 and 3) and 21°C (row 2 and 4). The first two columns represent females (left) and males (right) of the population from the Kasouga field site in South Africa. The last two columns represent individuals of the population from Semuliki National Park in Uganda. For each individual, we obtained 11 wing measurements corresponding to three categories of traits: ventral eyespots (1-3), ventral bands (4-5) and wing areas (6-7). Different letter codes were used to refer to the corresponding yellow rings (y), black discs (b) and white pupils (w). References to wing veins and cells follow the Comstock-Needham system (Miller 1970). The icons were provided by Manuel Marques-Pita and adjusted from Mateus et al. (2014).

Figure 2: Seasonal phenology of *Bicyclus safitza.* A) Monthly numbers of butterflies captured during the longitudinal survey in the Eastern Cape, South Africa (2014-2015) are given in $\log_2$-scale while the red dots represent monthly mean temperatures for a period of 30 years (1980-2009). In the temperate climate zone a flight period (Oct-Apr) was followed by a period in which no active adults were encountered (May-Aug). B) In tropical climate zones, here represented by data from Zomba in Malawi (1988-1989), adults of *B. safitza* are actively present throughout the year. Data presented in panel B were derived from Windig *et al* (1994).

Figure 3: The degree of plasticity for a suite of phenotypic traits in populations of *Bicyclus safitza* from temperate South Africa (blue) and tropical Uganda (red). Open symbols represent the degree of plasticity, calculated as the effects size (Hedges' g) between 21°C and 27°C, in females while males are represented by filled symbols. The black circles in the

legend at the bottom of the figure represent the effect size in Hedge's g. For details of the icons and codes for the wing pattern elements see Figure 1.

Figure 4: Effects of developmental temperature (A, B) and development time (C, D) on the first principal component (PC1) of nine ventral wing pattern elements (see Fig. 1) of *Bicyclus Safitza* butterfly. Panels A and C represent the data from the South African population whereas panels B and D represent the Ugandan population. Coloured dots represent the values for individuals reared at 21°C (purple), 23°C (blue), 25°C (pink) and 27°C (red). In graphs C and D, non-linear sigmoidal curves were fitted and explained over 49% and 80% of the variation in the population from South Africa and Uganda, respectively.

TABLES

Table 1: Variation in climatic conditions during a field survey of *Bicyclus safitza* in Eastern Cape, South Africa. Table shows air temperature (C °), relative humidity (RH %) and vapour pressure deficit (VPD kPa) range values and their standard deviations in three habitat types along the coastal range. Vapour-pressure deficit is the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated, and thus important in understanding the conditions under which an organism maintains its water balance during temperature changes.

Abiotic factor		min	mean	max	s.d.
Temperature					
	Open	11.05	21.50	43.54	4.80
	Edge	8.05	21.40	52.08	5.21
	Closed	9.10	20.49	39.60	3.90
Relative humidity					
	Open	12.59	77.80	100	16.75
	Edge	12.57	79.54	100	16.08
	Closed	17.25	84.16	100	13.45
Vapour pressure deficit					
	Open	0	0.71	7.41	0.82
	Edge	0	0.67	12.01	0.86
	Closed	0	0.45	6.00	0.51

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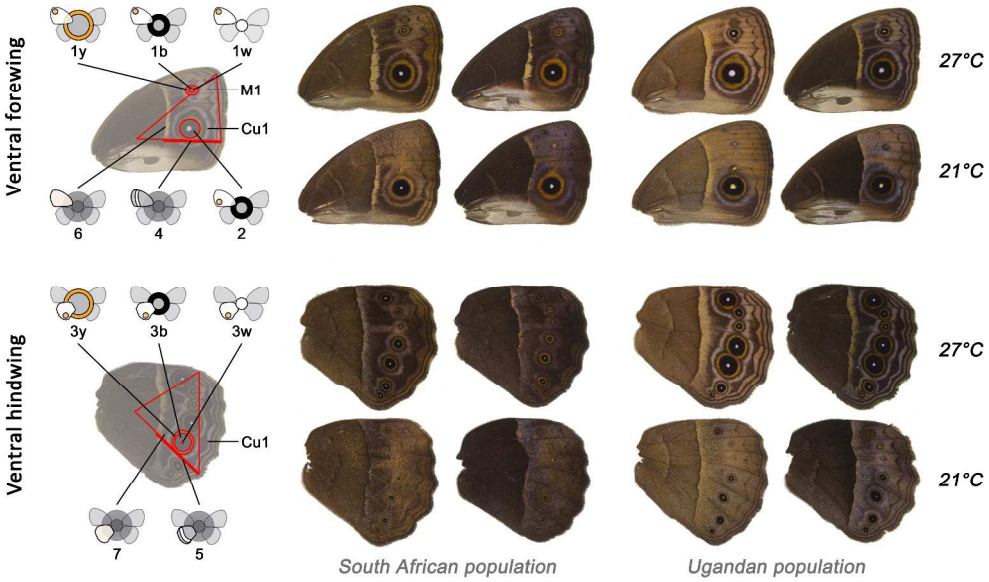

Figure 1: Wing pattern elements measured in all specimens of Bicyclus safitza. The images represent the typical phenotype of individuals reared at 27°C (row 1 and 3) and 21°C (row 2 and 4). The first two columns represent females (left) and males (right) of the population from the Kasouga field site in South Africa. The last two columns represent individuals of the population from Semuliki National Park in Uganda. For each individual, we obtained 11 wing measurements corresponding to three categories of traits: ventral eyespots (1-3), ventral bands (4-5) and wing areas (6-7). Different letter codes were used to refer to the corresponding yellow rings (y), black discs (b) and white pupils (w). References to wing veins and cells follow the Comstock-Needham system (Miller 1970). The icons were provided by Manuel Marques-Pita and adjusted from Mateus et al. (2014).

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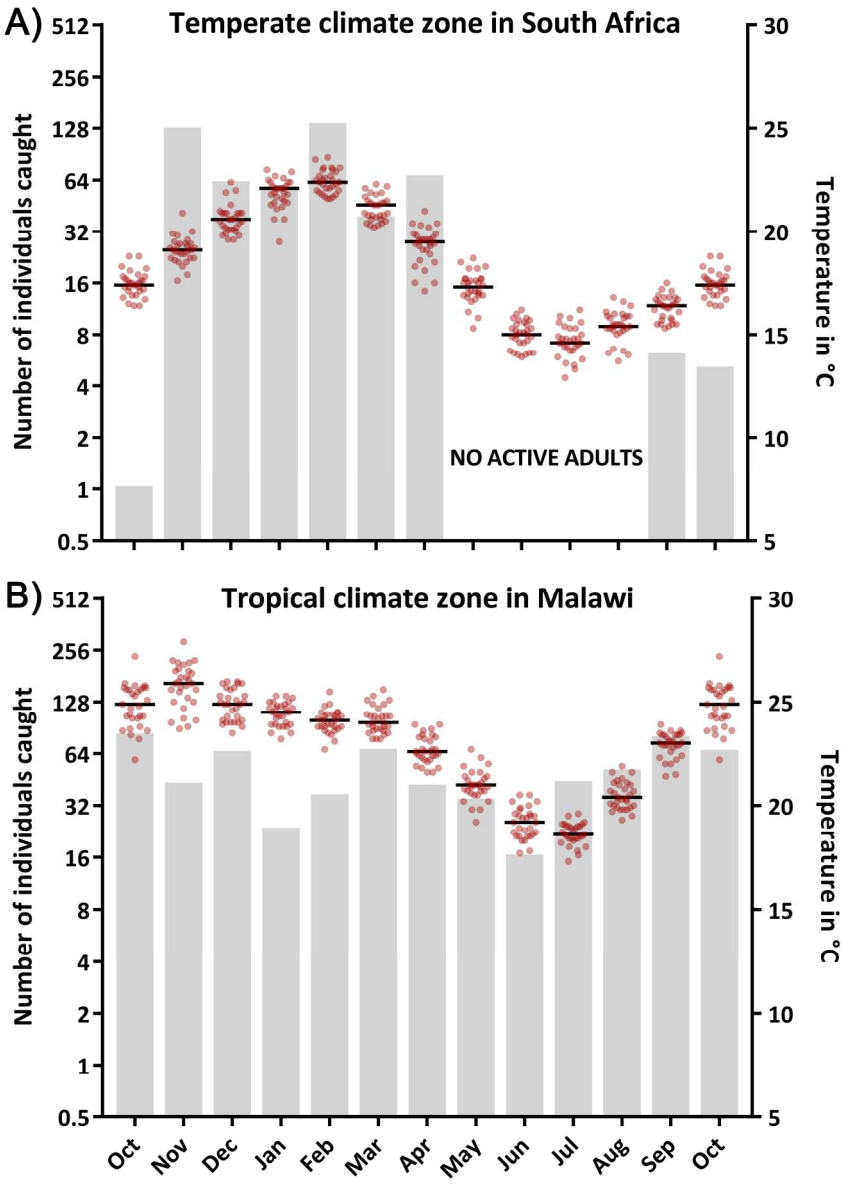

Figure 2: Seasonal phenology of Bicyclus safitza. A) Monthly numbers of butterflies captured during the longitudinal survey in the Eastern Cape, South Africa (2014-2015) are given in log2-scale while the red dots represent monthly mean temperatures for a period of 30 years (1980-2009). In the temperate climate zone a flight period (Oct-Apr) was followed by a period in which no active adults were encountered (May-Aug). B) In tropical climate zones, here represented by data from Zomba in Malawi (1988-1989), adults of B. safitza are actively present throughout the year. Data presented in panel B were derived from Windig et al (1994).

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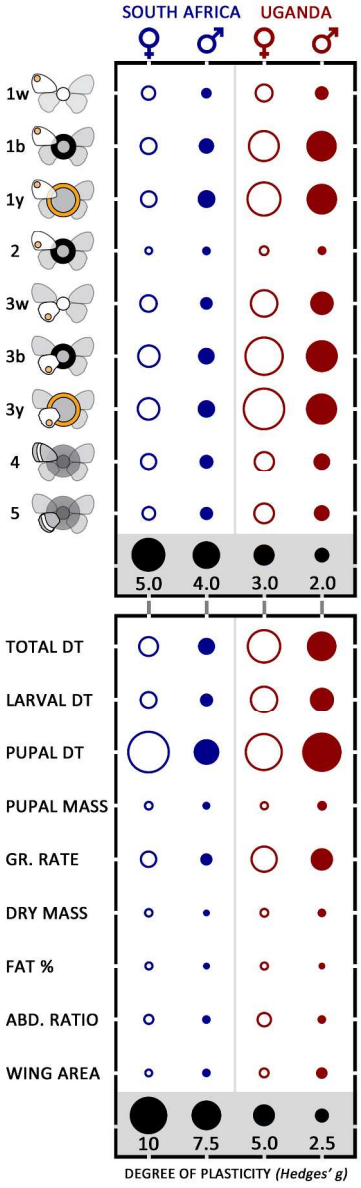

Figure 3: The degree of plasticity for a suite of phenotypic traits in populations of Bicyclus safitza from temperate South Africa (blue) and tropical Uganda (red). Open symbols represent the degree of plasticity, calculated as the effects size (Hedges' g) between 21°C and 27°C, in females while males are represented by filled symbols. The black circles in the legend at the bottom of the figure represent the effect size in Hedge's g. For details of the icons and codes for the wing pattern elements see Figure 1.

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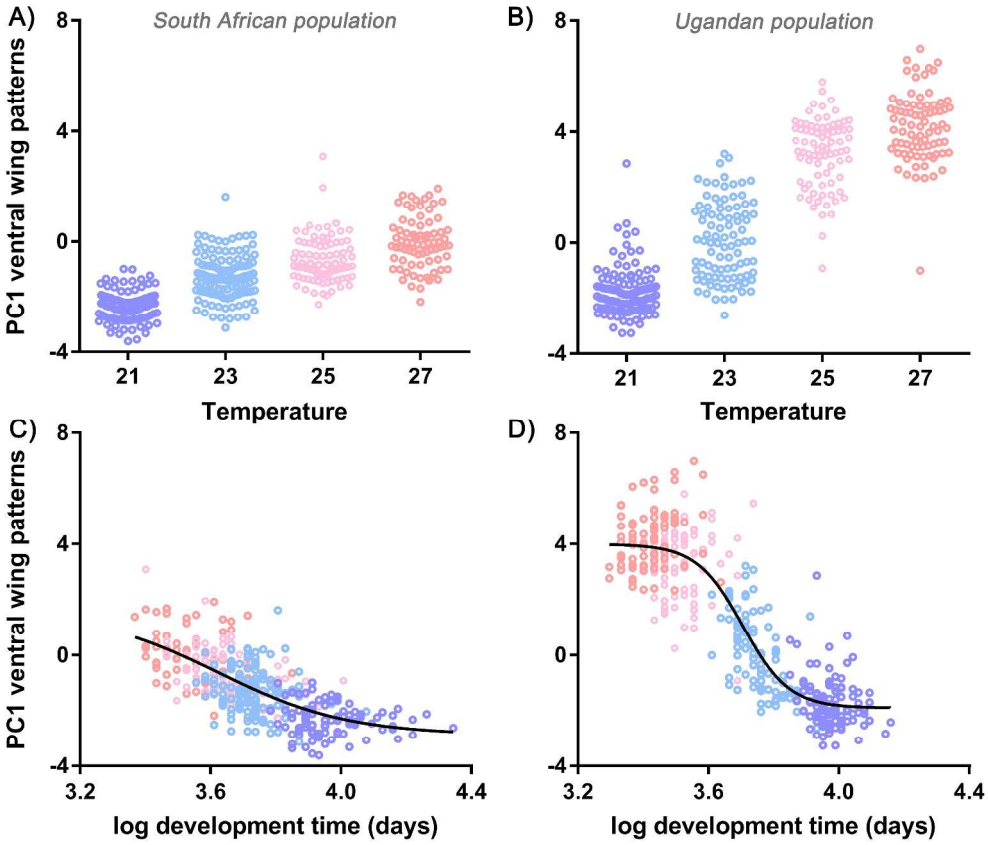


Figure 4: Effects of developmental temperature (A, B) and development time (C, D) on the first principal component (PC1) of nine ventral wing pattern elements (see Fig. 1) of Bicyclus Safitza butterfly. Panels A and C represent the data from the South African population whereas panels B and D represent the Ugandan population. Coloured dots represent the values for individuals reared at 21°C (purple), 23°C (blue), 25°C (pink) and 27°C (red). In graphs C and D, non-linear sigmoidal curves were fitted and explained over 49% and 80% of the variation in the population from South Africa and Uganda, respectively.

502x424mm (300 x 300 DPI)

Table 1 Minimum adequate models of the effect of population, developmental temperature and sex on a suite of phenotypic traits in populations of *Bicyclus safitza* from Uganda and South Africa, related to figure 3 in the main text and figure 1 in the supplementary material. Significant interactions between population and temperature are denoted in bold, indicating both populations respond significantly different to developmental temperature. The codes for the wing pattern element refer to figure 1 in the main text. Statistical significance is indicated as: * P <0.05, ** P <0.01, *** P <0.001. Data of the population from Uganda have previously been used in another study (van Bergen et al. 2017).

Trait	**(transformation)**	**fixed effects**	**F**	**df**	**df**	**P**
Total development time (d)	(log)	Population	51.3	1	715	***
		Temperature	1057.2	3	715	***
		Sex	27.3	1	715	***
		Population x Temperature	43.8	3	715	***
Larval development time (d)	(log)	Population	86.8	1	719	***
		Temperature	572.4	3	719	***
		Sex	49.7	1	719	***
		Population x Temperature	28.9	3	719	***
Pupal development time (d)	(log)	Population	68.7	1	715	***
		Temperature	2785.3	3	715	***
		Sex	100.3	1	715	***
		Population x Temperature	66.3	3	715	***
Pupal mass (mg)		Population	492.5	1	719	***
		Temperature	21.0	3	719	***
		Sex	518.5	1	719	***
		Temperature x Sex	4.9	3	719	**
Growth Rate (ln mg/d)	(log)	Population	190.5	1	719	***
		Temperature	459.8	3	719	***
		Sex	4.4	1	719	*
		Population x Temperature	37.4	3	719	***

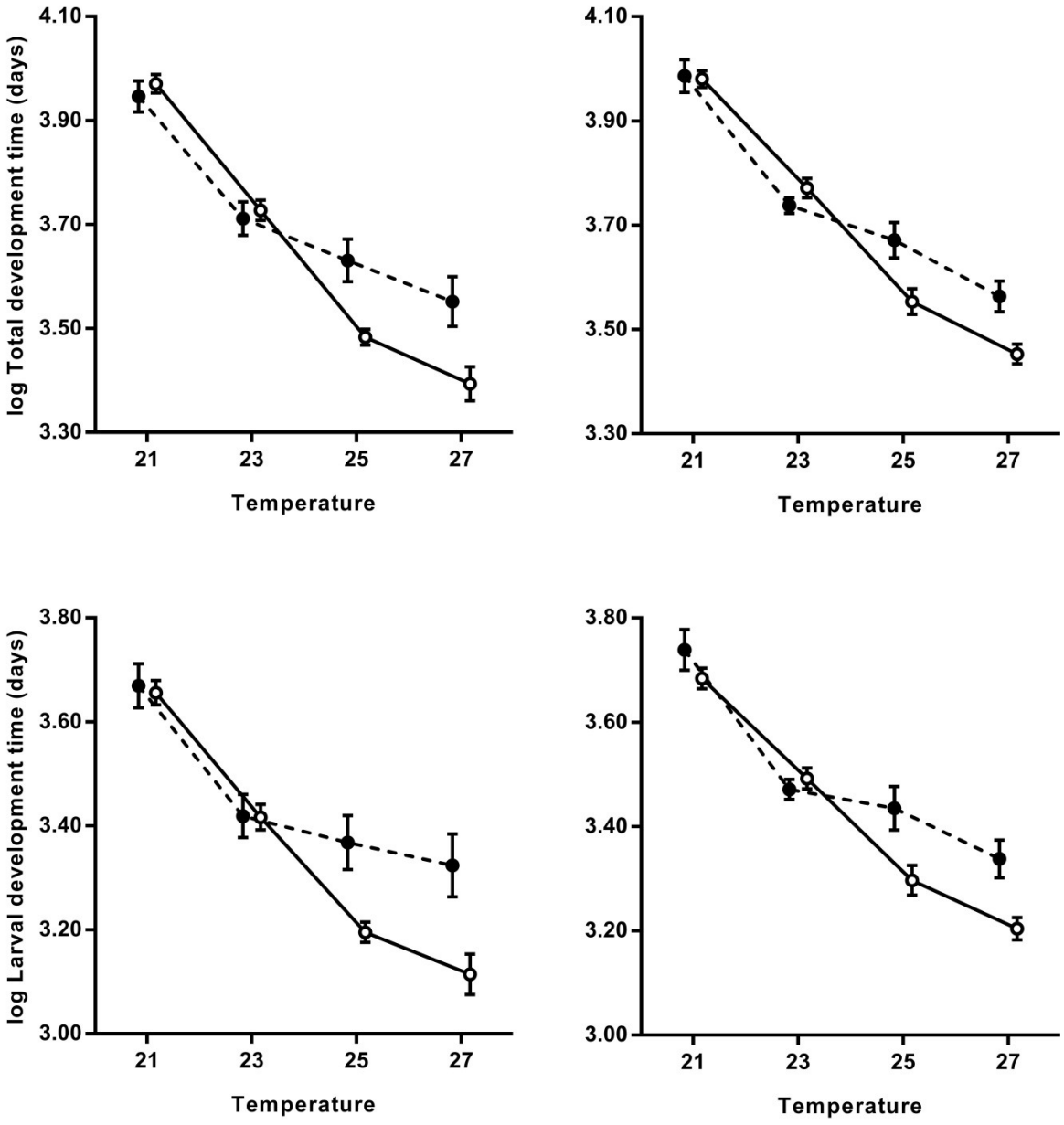
Table 1 *Continued*

Trait	**(transformation)**	**fixed effects**	**F**	**df**	**df**	**P**
Adult Drymass (mg)		Population	232.3	1	714	***
		Temperature	11.0	3	714	***
		Sex	1836.8	1	714	***
		Population x Sex	7.8	1	714	**
Adult Fat content (%)	(arcine)	Sex	127.8	1	713	***
Abdomen Ratio (%)	(arcine)	Population	9.5	1	712	**
		Temperature	35.2	3	712	***
		Sex	3082.7	1	712	***
		Population x Temperature	3.4	3	712	*
Total Wing Area (mm^2)		Population	1299.6	1	710	***
		Temperature	25.4	3	710	***
		Sex	1384.5	1	710	***
		Population x Temperature	4.6	3	710	**
		Population x Sex	4.1	1	710	**
Wing pattern element 1y	(size corrected)	Population	135.1	1	715	***
		Temperature	363.2	3	715	***
		Population x Temperature	88.0	3	715	***
Wing pattern element 1b	(size corrected)	Population	490.1	1	714	***
		Temperature	325.2	3	714	***
		Sex	4.5	1	714	***
		Population x Temperature	113.2	3	714	***
Wing pattern element 1w	(size corrected)	Population	605.3	1	713	***
		Temperature	119.4	3	713	***
		Sex	7.5	1	713	**
		Population x Temperature	26.2	3	713	***
		Population x Sex	7.4	1	713	**
Wing pattern element 2	(size corrected)	Population	188.5	1	716	***
		Temperature	29.8	3	716	***
		Sex	100.1	1	716	***
		Population x Sex	16.1	3	716	***

Table 1 *Continued*

Trait	**(transformation)**	**fixed effects**	**F**	**df**	**df**	**P**
Wing pattern element 3y	(size corrected)	Population	776.2	1	717	***
		Temperature	490.2	3	717	***
		Sex	4.0	1	717	*
		Population x Temperature	113.6	3	717	***
Wing pattern element 3b	(size corrected)	Population	1197.9	1	718	***
		Temperature	447.7	3	718	***
		Population x Temperature	145.9	3	718	***
Wing pattern element 3w	(size corrected)	Population	1456.8	1	716	***
		Temperature	335.9	3	716	***
		Sex	16.0	1	716	***
		Population x Temperature	168.6	3	716	***
		Population x Sex	6.0	1	716	*
Wing pattern element 4	(size corrected)	Population	214.3	1	716	***
		Temperature	175.3	3	716	***
		Sex	467.6	1	716	***
		Population x Sex	4.2	1	716	*
Wing pattern element 5	(size corrected)	Population	18.7	1	719	***
		Temperature	146.4	3	719	***
		Sex	192.5	1	719	***
		Population x Sex	9.8	1	719	**

Figure 1 Reaction norm representations of the effect of population, developmental temperature and sex on a suite of phenotypic traits in populations of *Bicyclus safitza* from Uganda and South Africa, related to figure 3 in the main text and table 1 in the supplementary material. Data from males is given in the left hand panel, females in the panel on the right. The South African population is represented by the filled symbols and dashed lines. Ugandan population is represented open symbols and solid lines Codes for the wing pattern element refer to figure 1 in the main text and error bars represent 95% confidence intervals.



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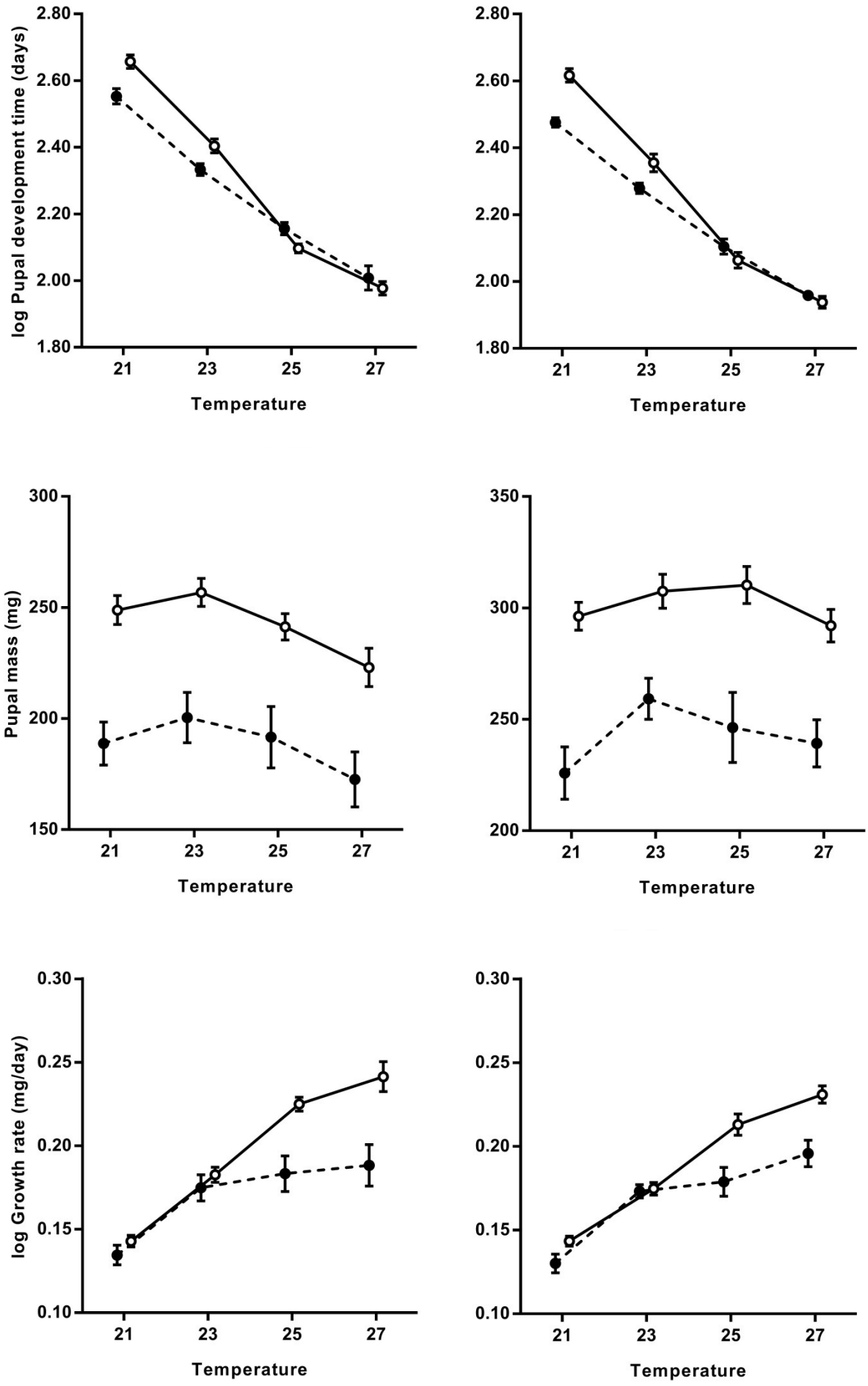

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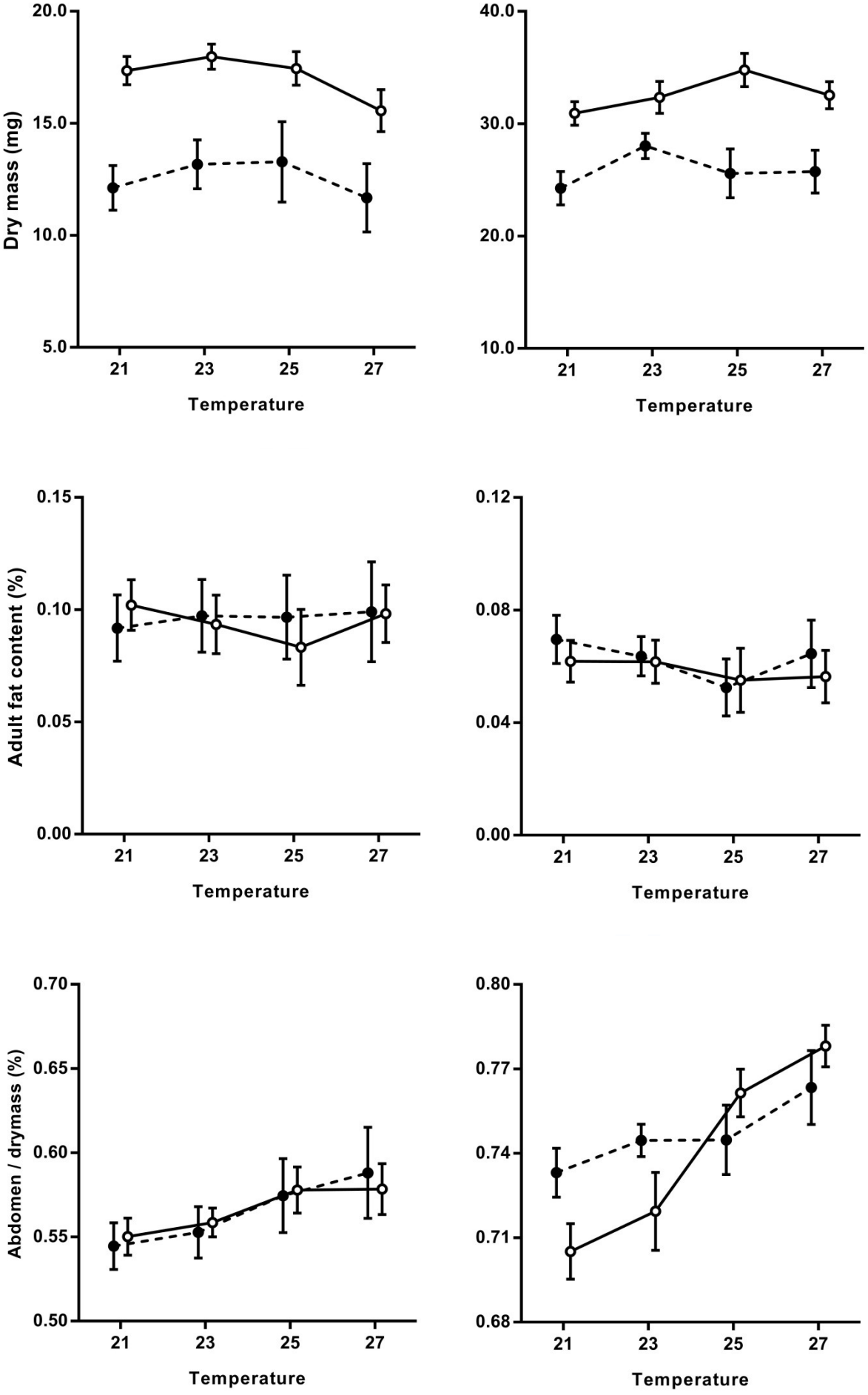

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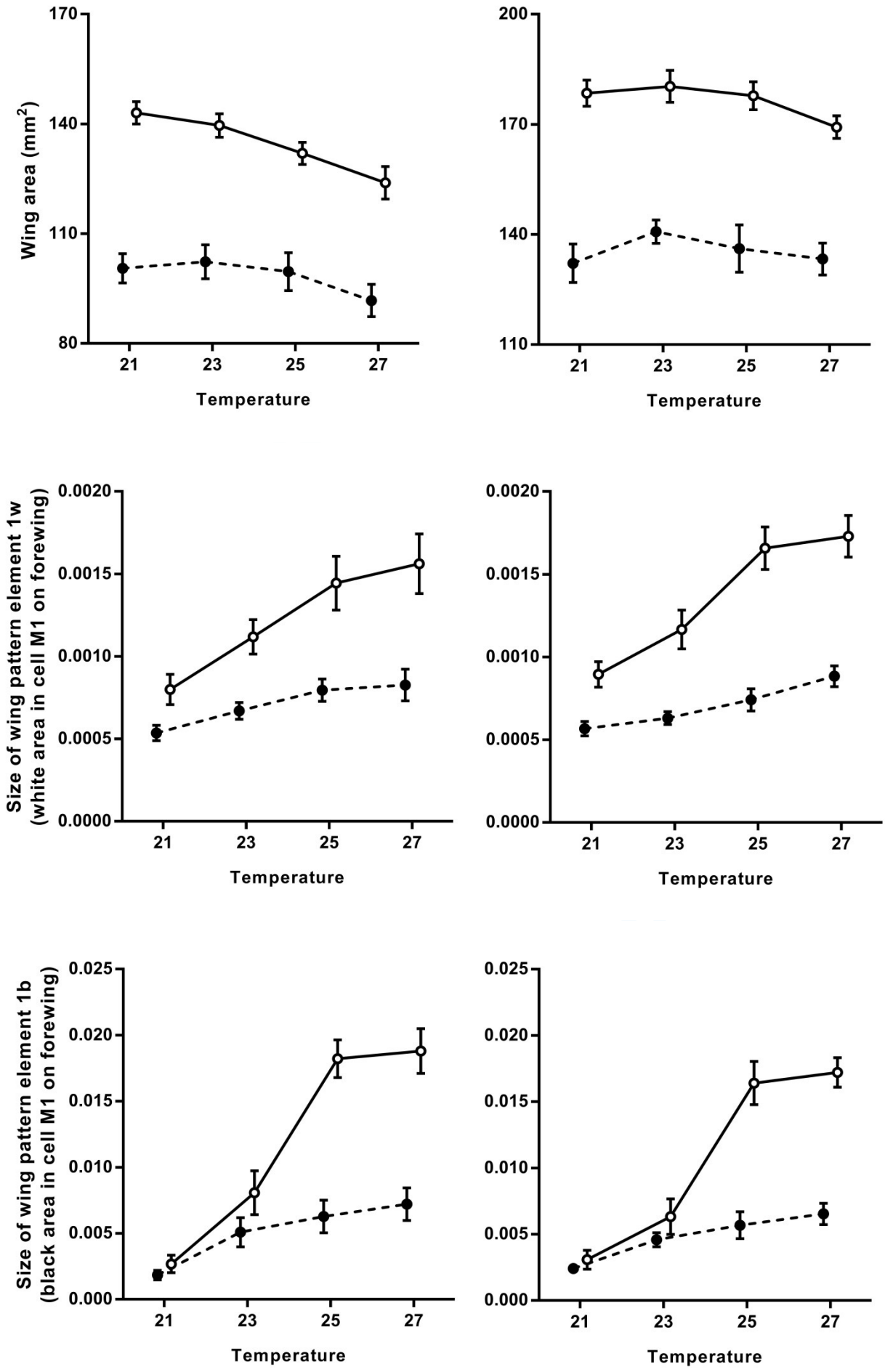

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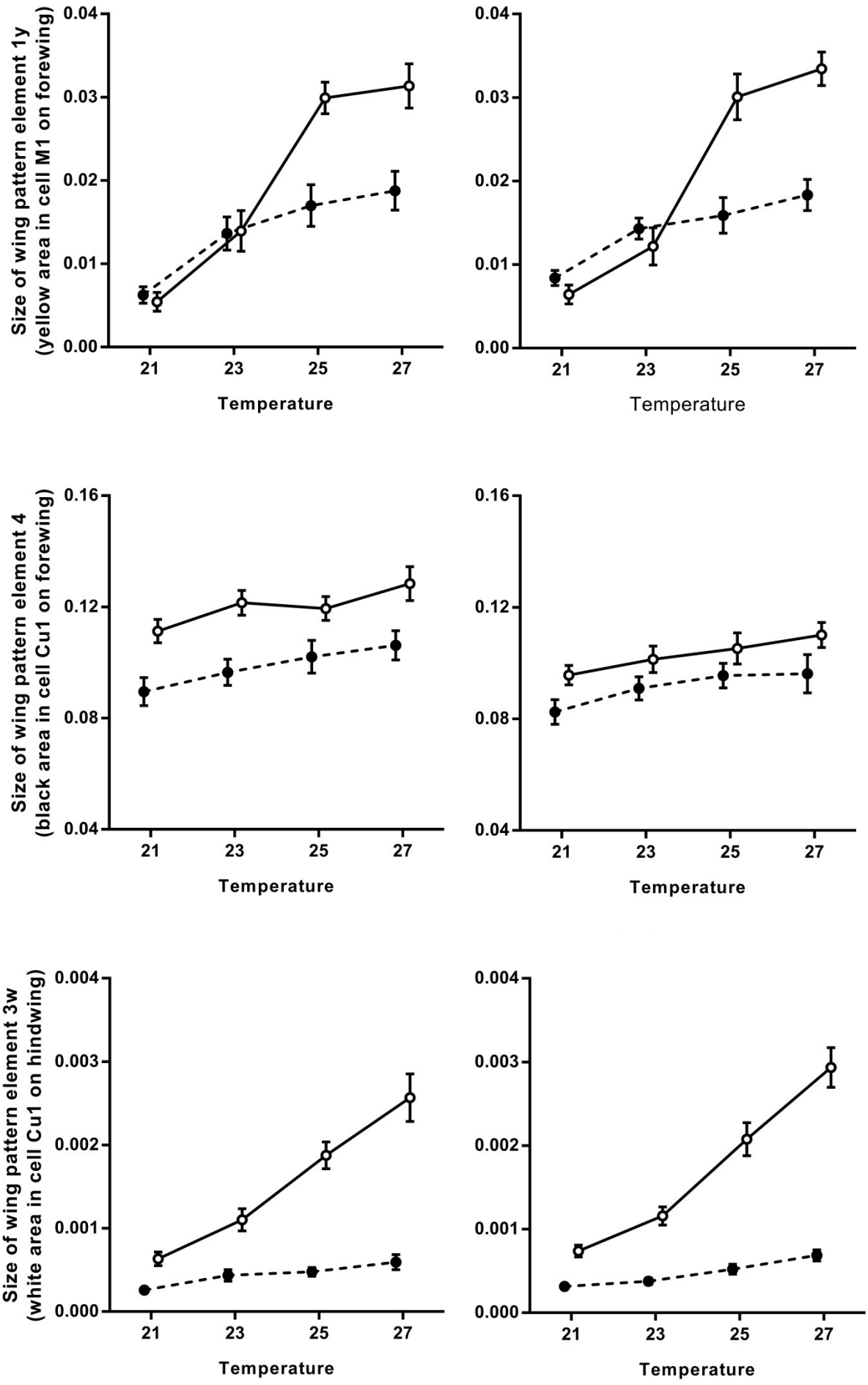

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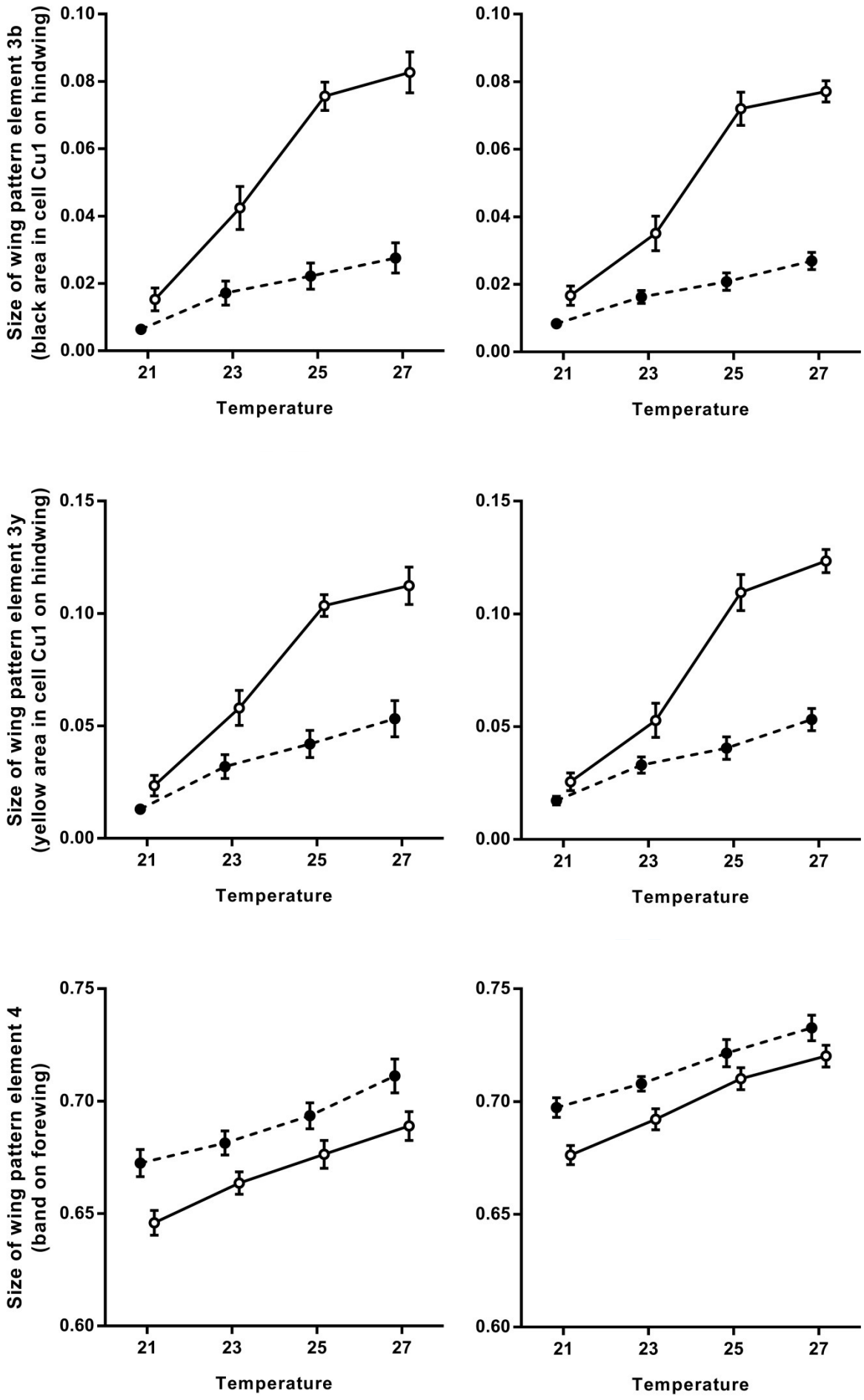

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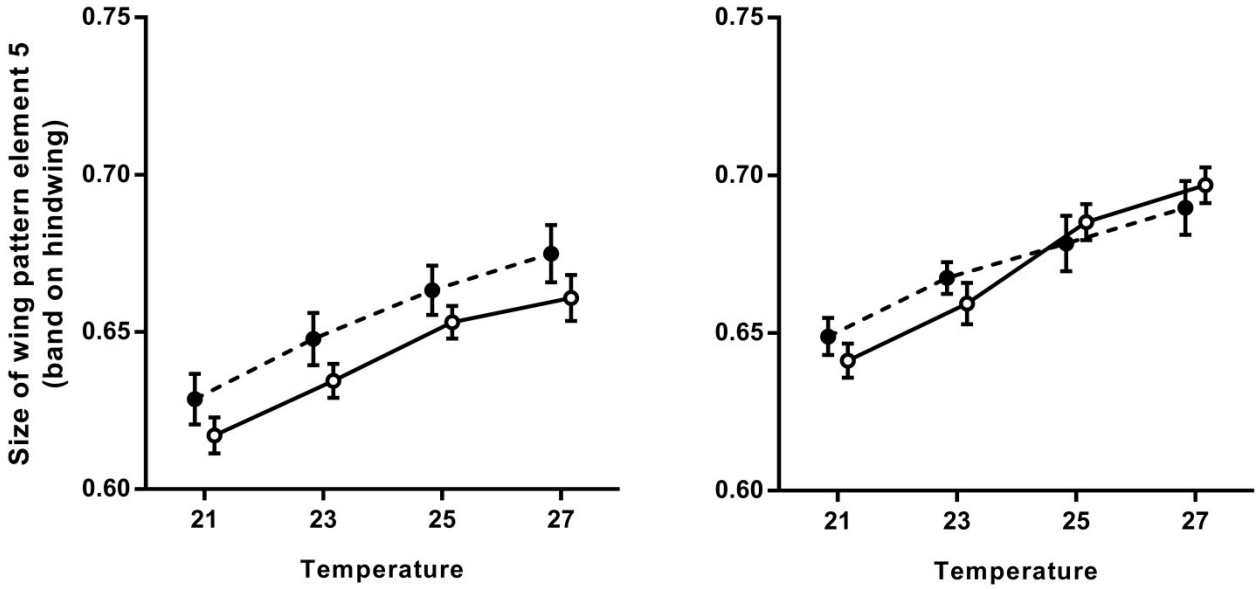

Table 2: Extrapolated larval water loss of *Bicyclus safitza* in Eastern Cape, South Africa. Water loss was calculated as a percentage of larval body mass per hour (% H_2O g^{-1} hr^{-1}). Negative values indicate water uptake (i.e. weight gain), while positive values indicate water (or weight) loss in relation to body weight (i.e. 100 would indicate the loss of entire body weight within an hour).

Larval water loss		min	mean	max	s.d.
Habitat					
	Open	-10.46	-5.11	38.52	5.36
	Edge	-11.11	-5.38	68.47	5.66
	Closed	-10.44	-6.80	29.35	3.36
Site					
	Kapriver	-10.46	-4.62	68.47	6.66
	Kasouga	-11.11	-6.69	16.53	2.89
	Bathurst	-10.44	-6.52	24.84	3.99
Month					
	November	-9.79	-5.79	5.06	3.13
	December	-10.11	-6.04	19.14	4.20
	January	-10.45	-5.28	30.90	5.27
	February	-11.11	-6.24	35.09	4.45
	March	-10.51	-5.75	68.47	5.90
	April	-10.46	-6.08	27.47	4.12

*Negative values imply water uptake and positive values water loss.