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Climatic factors shape plastic trade-offs in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae)

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Abstract: Aim Trade-offs allow individuals to optimize their fitness by tailoring the investment into different traits to variable environmental conditions, such as along geographical gradients. Trade-offs thus can help in adjusting to changing thermal and insolation profiles, especially in small ectotherms, whose body temperature typically follows environmental temperatures closely. Two traits usually involved in latitudinal adaptation are body size and melanism. Since both traits are costly, individuals need to optimize investment into each trait. Here, we studied how environmental factors influence this trade-off in the short and long-term. Location Europe. Methods We raised flies from 15 latitudinal populations at three constant temperatures in a laboratory common garden to differentiate plastic and evolutionary responses to temperature. We further analysed how the different insolation components of the populations' habitats influenced the evolution of the trade-off. Results Male *Sepsis thoracica* (Diptera: Sepsidae) feature a sigmoid relationship between melanism and body size, defining two strikingly different male morphs: obsidian (small and black) and amber (large and orange). This relationship was altered by the developmental temperature, documenting its plasticity. The relationship further evolved across populations in response to the environmental characteristics of their habitat, notably temperature, insolation and UV radiation, suggesting that plasticity also has an underlying genetic basis. Nevertheless, melanism, but not body size, merely slightly increased with latitude. Main conclusions As the plastic and evolutionary responses of the relationship to temperature differed, plasticity does not necessarily follow the direction of evolution of this trade-off, but rather adds to it. Our study evinces the role of several environmental factors in shaping the evolution of a plastic melanism—body size relationship defining a rare male polymorphism in temperate sepsid flies.

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Climatic factors shape plastic trade-offs in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae)

SHORT RUNNING HEAD: Climatic factors shape plastic biological relationships

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colouration, body size, insolation, long-wave radiation, latitude, melanism, plasticity, short-wave radiation, temperature

19 **ABSTRACT**

20 **Aim**

21 Trade-offs allow individuals to optimize their fitness by tailoring the investment into different traits to
22 variable environmental conditions, such as along geographic gradients. Trade-offs thus can help in
23 adjusting to changing thermal and insolation profiles, especially in small ectotherms, whose body
24 temperature typically follows environmental temperatures closely. Two traits usually involved in
25 latitudinal adaptation are body size and melanism. Since both traits are costly, individuals need to
26 optimize investment into each trait. Here we studied how environmental factors influence this trade-off
27 in the short and long term.

28 **Location**

29 Europe

30 **Methods**

31 We raised flies from 15 latitudinal populations at three constant temperatures in a laboratory common
32 garden to differentiate plastic and evolutionary responses to temperature. We further analysed how the
33 different insolation components of the populations' habitats influenced the evolution of the trade-off.

34 **Results**

35 Male *Sepsis thoracica* (Diptera: Sepsidae) feature a sigmoid relationship between melanism and body
36 size, defining two strikingly different male morphs: obsidian (small and black) and amber (large and
37 orange). This relationship was altered by the developmental temperature, documenting its plasticity.
38 The relationship further evolved across populations in response to the environmental characteristics of
39 their habitat, notably temperature, insolation and UV radiation, suggesting that plasticity also has an
40 underlying genetic basis. Nevertheless, melanism, but not body size, merely slightly increased with
41 latitude.

42 **Main Conclusions**

43 As the plastic and evolutionary responses of the relationship to temperature differed, plasticity does not
44 necessarily follow the direction of evolution of this trade-off, but rather adds to it. Our study evinces the
45 role of several environmental factors in shaping the evolution of a plastic melanism – body size
46 relationship defining a rare male polymorphism in temperate sepsid flies.

INTRODUCTION

Trade-offs allow individuals to optimize their fitness in different environments by adjusting the relative investment into various traits to the environmental conditions. This differential investment allows species to extend their ranges, for example over a wide latitudinal gradient (Fleming & Gross, 1990; Ardia, 2005; Laurila *et al.*, 2008). Along any geographic gradient, organisms have to adapt to various climatic factors including season length, thermal profiles and insolation regimes. This can be achieved by plastically or genetically adjusting trade-offs in order to optimize the phenotype to the particular set of environmental conditions of their local habitat (Abraham & Jain, 2005). Such adaptation to environmental factors is of major importance particularly for small ectothermic organisms, such as insects, whose body temperature typically follows environmental temperatures closely (Buckley *et al.*, 2012). Though there are many exceptions, increased temperature usually results in a body size decrease in ca 80% of all inspected arthropod species (temperature-size rule: Atkinson, 1994; Klok & Harrison, 2013; Horne *et al.*, 2015), providing one way by which body size is adjusted to different thermal profiles. While this decrease in size is entirely plastic, body size divergence among geographic or latitudinal populations is often genetic (Partridge & Coyne, 1997; Chown & Gaston, 2010). Whereas cooler temperatures at higher latitudes often result in positive Bergmann size clines (larger towards the poles), season length decreases with latitude can constrain an individual's growth and hence final body size, resulting in converse Bergmann size clines especially in large species with long development (smaller towards the poles: Blanckenhorn & Demont, 2004; Nygren *et al.*, 2008). Thus, latitude can influence ectotherm body size through various climatic factors and mechanisms.

Besides temperature and season length, insolation also changes drastically with latitude. The total daily solar energy decreases with latitude, and parallel to this insolation decrease many insects show increased melanism (reviewed by Clusella Trullas *et al.*, 2007). Darker pigmentation facilitates absorption of the extra sunlight and helps increase the insect's body temperature (Watt, 1969; Lindstedt *et al.*, 2009).

Thus darker individuals can increase their performance at cooler conditions by being more active at suboptimal temperatures (Ellers & Boggs, 2002; Clusella Trullas *et al.*, 2007). It follows that melanism is likely to evolve from originally non-melanic ancestor traits particularly in geographic regions with limited sunlight. As with body size, pigmentation differentiation among populations of different latitudes can be genetic (David *et al.*, 1985).

The aforementioned predicts that most insects should show increased body size and melanism with latitude. However, both body size (Reichle, 1968) and pigmentation are resource-demanding traits, as melanin is costly to synthesize (Talloen *et al.*, 2004) and its synthesis further produces cytotoxic by-products (Schmid-Hempel, 2003; González-Santoyo & Córdoba-Aguilar, 2012). An organism with a widespread latitudinal distribution therefore will need to balance the allocation of resources between body size and melanism in optimizing its phenotype to the particular thermal and insolation conditions at each location.

An excellent system to address how climatic factors influence the relationship between body size and pigmentation is *Sepsis thoracica* (Robineau-Desvoidy, 1830; Diptera: Sepsidae). Sepsids are a family of acalyptrate flies generally associated with decaying organic matter with, and with ca. 300 species worldwide. Most species are shiny-black, but amber colour recurs repeatedly throughout the phylogeny, and at least in the *Sepsis* clade common in Europe, amber colour may well be the ancestral character state (and melanism derived; Ang *et al.*, 2013: see <http://sepsidnet-rmbr.nus.edu.sg/> for pictures of many species). In Europe, *S. thoracica* is the only *Sepsis* species that can be entirely amber, a colouration that is more commonly observed in tropical species. Moreover, whereas *S. thoracica* females are always totally black, males of this species exhibit extreme variability in size and colour, with smaller individuals being black and large individuals being bright amber (Pont & Meier, 2002; see pictures in Fig. 3). This suggests a trade-off between pigmentation and body size, as the relationship is steeply sigmoid (Busso *et al.*, 2017), a situation that may occur in other related temperate species (e.g. in *Saltella sphondylii*), but to date no

studies of colouration in any sepsid are available. The fact that the variation is restricted to males suggests a role of sexual selection (treated elsewhere in Busso, 2017). Because of its wide European range, *S. thoracica* permits comparison of different populations across a geographic (latitudinal) gradient, amber and black individuals being present in the field in unknown proportions in all populations, thus providing a unique opportunity to analyse how the environment influences the relative resource allocation to two costly phenotypic traits, here body size and melanism.

Since *S. thoracica* is a small species with short development time, we hypothesized that it would follow the temperature-size rule, as do other insects with similar life histories, and be subject to few seasonal time constraints (Atkinson, 1994; Blanckenhorn & Demont, 2004; Berger *et al.*, 2013). For the same reason, we expected that the average temperature decrease would mediate increased body size with latitude (Bergmann cline), accompanied by more amber phenotypes if there is indeed a trade-off. Alternatively, if insolation is more important, we hypothesized that reduced insolation at higher latitudes would intensify melanisation, accompanied by smaller body size in case of a trade-off. We raised flies from 15 European populations at three constant temperatures in a laboratory common garden experiment to differentiate plastic and evolutionary responses to temperature. By further considering the climatic conditions of the populations' habitats from data banks, we attempted to uncover how the local environment influences the evolution of the relationship between body size and melanism.

MATERIALS AND METHODS

Population sampling and fly culture maintenance

We sampled flies from 15 European *S. thoracica* populations along a latitudinal gradient: Pehka, Estonia (59.48° N, 26.37° W); Aarna, Estonia (58.07° N, 26.97° W); Ludwigshafen, Germany (49.48° N, 8.42° W); Nordrach, Germany (49.4° N, 8.08° W); Zurich, Switzerland (47.34° N, 8.54° W); Borgonuovo,

Italy (46.33° N, 9.44° W); Capriasca, Switzerland (46.07° N, 8.97° W); Asturias, Spain (43.3° N, 6.0° E);
 Petroia, Italy (43.23° N, 12.56° W); Terni, Italy (42.57° N, 12.62° W); Prati di Stronconi, Italy (42.48° N,
 12.68° W); Viggiano, Italy (40.34° N, 15.81° W); Padula, Italy (40.32° N, 15.67° W); Lamezzia, Italy
 (38.92° N, 16.25° W); and Adrano, Italy (37.67° N, 14.83° W). Wild-caught females were brought to the
 laboratory and used to establish cultures of multiple (5–25) replicate iso-female lines per population (i.e.
 families: the offspring of one field-caught female), which were housed in separate plastic containers and
 regularly supplied with fresh cow dung, sugar, and water ad libitum. The cultures were, somewhat
 arbitrarily and for experimental convenience, maintained at 18 ± 1 °C under a 14:10 h light:dark cycle.

Rearing latitudinal lines

To analyse how environmental factors shape the relationship between body size and melanism,
 we reared multiple lines (2-10) from the various populations in a laboratory common garden. A small
 container with more or less dung, to obtain a representative sample of individuals for each family
 through larval food competition, was placed for 24 hours inside any of the *S. thoracica* stock cultures for
 females to deposit eggs. This dung was incubated afterwards in a novel container in climate chambers at
 60% humidity, and 14:10 h light:dark cycle to obtain adult flies. Flies were reared at three constant
 temperatures: 18 °C, 24 °C, 30 °C. This method of using iso-female lines instead of wild-caught females
 in principle permits separation of quantitative genetic (not treated here), plastic and environmental
 influences on body size and melanism. Since sepsid larvae and pupae develop within the dung pats into
 which the females laid their eggs, insolation will not reach them directly, but will influence the
 temperature of the dung pat. Because of this indirect influence through temperature, we decided to
 focus on the effects of temperature during development.

Morphometric measurements

Emerging individuals were dissected and the outer, dorsal side of their fore femur was photographed under a stereo microscope MZ12 with a DFC490 camera (Leica) against a neutral white background (cf. Fig. 3). The camera was calibrated with a mini IT-8 calibration target, to guarantee colour consistency among the pictures. The target also served as a scale to later measure size. We used a self-written code in ImageJ, which measured the number of pixels of the fore femur and converted them to the real area according to the scale corresponding to each picture. The code also estimated the number of melanic pixels in the femur, guaranteeing an objective quantification of melanism. Any pixel with a V-value over 163 in the YUV colour space was defined as melanic, corresponding to the valley in the bimodal V-value distribution of femur colouration (Y: brightness; U: blue-luminance; V: red-luminance; see <https://en.wikipedia.org/wiki/YUV>; cf. Fig. 3, right). Melanism represented the proportion of black and brown pixels over all the pixels of the femur (thus requiring a logistic approach). Colouration and body size covary in all body parts of *S. thoracica* males. For a sample of $n = 204$ males of various sizes, the correlations in the melanism of their fore femur, hind femur and thorax (scutum) were very high ($r > 0.95$), verifying that the widened fore femur, a secondary sexual trait in many sepsids, well represents overall colour (head and abdomen were not scored for technical reasons). Body size was expressed as the total area of the male's fore femur (square-root-transformed to linearize the measure in some analyses). Bivariate linear correlations among all body parts exceed $r > 0.95$.

Environmental variables

The thermal and insolation profiles of the various locations were obtained from the ECMWF's ERA-Interim database (Dee *et al.*, 2011). We retrieved all the main variables related to temperature and insolation available in the dataset. For each location we obtained the mean temperature and insolation. The latter was decomposed into its different components: surface solar radiation downwards (short-wave direct and diffuse radiation that reaches the Earth's surface), surface net solar radiation (net short-

wave radiation after subtracting the part reflected by the earth), surface thermal radiation downwards (long-wave radiation reaching the Earth's surface), and downward UV radiation at the surface (UV radiation reaching the Earth's surface). We then averaged the values between 1979 and 2016 for the warmest months (May to October) and used these averages as predictor variables in the models described below. We additionally considered latitude and the length of the season as the number of days in which the mean temperature remained above 9 °C (cf. Blanckenhorn, 1999).

Statistical analyses

The males' multimodal distribution in melanism with two valleys was used to determine threshold values for the separation of the two male morphs (Fig. 3): obsidians (small and black) and amber (large and orange). We also found some intermediate phenotypes, and consequently defined three levels of the factor *morph* in the following models.

To determine if latitude influenced body size and melanism we fitted two separate models, one for body size (raw and square-root-transformed) and one for melanism. For body size we fitted a linear mixed effect model with latitude, developmental temperature, and morph plus all two-way interactions between them (N = 2998 individual males). For melanism, a proportional response variable, we used an analogous logistic generalized mixed effect model. We also incorporated in all models a random effect of line nested within population to control for genetic relatedness. We always used the 'lme4' package of the software R Version 3.2.2 (R Development Core Team, 2015).

To first analyse the general relationship between body size and melanism in *S. thoracica* males only, we fitted the five-parameter logistic regression model

$$\text{Melanism} = c + (d-c)/[1+(BS/e)^b]^f,$$

where BS is body size, d is the estimated melanism at body size zero, c is the estimated melanism at infinite body size, e is the mid-range body size, b is the slope factor, and f is the asymmetry factor (Gottschalk & Dunn, 2005). This regression was independently fitted to each iso-female line of each population at every developmental temperature, to determine if the sigmoid curves differ significantly between populations. We studied a total of 98 iso-female lines, which rendered it difficult to achieve a fully factorial design (with all lines at all temperatures) due to some lines dying in the process. Nevertheless, all populations were represented by at least three lines at all temperatures. We further calculated the derivative of each curve to obtain the maximum slope at the inflection point, yielding the body size at which the curve changes from concave to convex. It is worth noting that, mathematically, the maximum slope is not the same as the slope factor, and the inflection point is not the same as the mid-range body size. From each population fit we calculated the residuals, which were then squared and added to obtain a sum of squares that represented the overall error (SS_{error}). We similarly added the degrees of freedom of all the population models to obtain the degrees of freedom of the error (df_{error}). Then we fitted the five-parameter logistic regression to the entire male dataset ($N = 2998$) to evaluate the general model. We squared and added the residuals of the general model to obtain the total error (SS_{total}), and calculated an F-statistic with the following formula:

$$F = \frac{(SS_{total} - SS_{error}) / (df_{total} - df_{error})}{(SS_{error} / df_{error})}.$$

The F -value and the degrees of freedom were used to calculate the P -value of the population differences. These calculations were necessary because a regular ANOVA does not allow fitting a five-parameter logistic regression to the data.

To analyse the effects of temperature and insolation on the body size – melanism trade-off, we fitted a model to each of the five parameters of the logistic regression as outcome variables. Each model included the developmental temperature and all above-mentioned environmental variables, which are

population characteristics. Interactions between developmental temperature and the environmental variables were first entered but later removed from the models because they were always non-significant. We used the R-package 'lme4' to test the significance of each variable in each model. Since the model only investigates 15 populations and the environmental variables are population-specific, this did not leave much power to analyse interactions between all environmental variables. We again also incorporated in all models line nested within population as random effects.

RESULTS

Effect of rearing temperature and latitude on body size and melanism

Body size (square-root-transformed fore femur area) was negatively affected by developmental temperature in accordance with the temperature-size-rule (Fig. 1; $X_1^2 = 266.42$, $P < 0.001$) and differed significantly between the *morphs* (Fig. 1; $X_2^2 = 6115.84$, $P < 0.001$). The interaction between temperature and morph was also significant (Fig. 1; $X_2^2 = 66.68$, $P < 0.001$). Latitude showed no significant main or interactive effects on body size (*latitude*, $X_1^2 = 0.54$, $P = 0.463$; *latitude:temperature*, $X_1^2 = 3.42$, $P = 0.074$; *latitude:morph*, $X_2^2 = 1.42$, $P = 0.491$). By contrast, melanism slightly increased with latitude overall (Fig. 2; estimate = 0.105 ± 0.095 , $X_1^2 = 6.33$, $P = 0.012$), with developmental temperature and the interaction between the two variables showing no effect (*temperature*, $X_1^2 = 0.351$, $P = 0.554$; *latitude:temperature*, $X_1^2 = 0.017$, $P = 0.898$).

Body size – melanism trade-off

We found a sigmoid relationship between body size and melanism in *S. thoracica*, which was present in all 15 sampled populations and differed significantly between populations ($F_{85,2835} = 6.566$, $P < 0.001$). While the body size distribution was unimodal, melanism showed a trimodal distribution with two valleys (Fig. 3), which we used post-hoc to classify the morphs of the species. This yielded the

following threshold values for the separation of the males into morphs: the first valley in melanism at 0.73 marked the lower limit of the obsidian morph, and the second valley at 0.43 marked the upper limit of the amber morph. The remaining males were classified as intermediate (Fig. 3).

Effect of developmental temperature and environmental variables on the trade-off

Several environmental variables significantly influenced some of the five parameters defining the shape of the logistic regression curve between body size and melanism in male *Sepsis thoracica* (Table 1). To assist in their interpretation, the changes in the mean sigmoid curves (based on the entire dataset) resulting from a one standard deviation (SD) increase in the respective significant parameters are plotted for these environmental variables (Fig. 4). Figure 5 shows the actual curve differences between the two most extreme latitudinal populations.

DISCUSSION

Our study of geographic variation in body size and colouration across 15 latitudinal *Sepsis thoracica* (Diptera: Sepsidae) populations in Europe yielded several salient results. While females are always all black, males display a sigmoid relationship between body size and melanism, defining a body size threshold that divides males into two colour morphs: obsidian (small and black) and amber (large and orange; Fig. 3), whose body sizes differ greatly (Fig. 1). Body size diminished with developmental temperature, defining the minimal null expectancy of temperature plasticity (Fig. 1). Contrary to initial expectation, body size did not change with latitude (no cline), but populations showed heritable geographic variation in response to several climatic factors (Figs. 4 & 5; Table 1). By contrast, *S. thoracica* males on average became slightly but significantly darker (i.e. more melanic) with latitude, in agreement with our initial expectation. The sigmoid relationship between body size and melanism was affected in complex ways by some of the environmental habitat characteristics considered (Figs. 4, 5),

but not others (including season length), from which we conclude that temperature and insolation have played complementary roles in shaping the trade-off relationship depending on geographic location.

Above a certain body size threshold *S. thoracica* males dramatically reduce investment in their black cuticular pigmentation (which covers the underlying colour), becoming almost completely amber (Fig. 3). Busso *et al.* (2017) demonstrated a role of insect immunity in shaping this relationship, suggesting a trade-off between being small and black (with high phenoloxidase levels conferring better immune defence against parasites) or large and amber (conferring greater mating success; Busso, 2017). Amber colouration is common in sepsid flies (Pont & Meier, 2002; Ang *et al.*, 2013), perhaps even the ancestral character state in some clades (see Introduction). We suggest that melanism, plastic or fixed, commonly evolved in multiple temperate sepsid lineages after colonizing cooler habitats (cf. Clusella Trullas *et al.*, 2007). Polymorphisms similar to those of *S. thoracica* therefore may well occur in related species (e.g. *Saltella sphondylii*, *Australosepsis spp.*), implying more general evolutionary relevance, but so far ours is the first study of colouration in sepsid flies. Threshold traits as observed here (Fig. 1) and e.g. in the necrophagous fly *Prochyliza nigrimana* (Diptera: Piophilidae: Martin-Vega & Baz, 2011), the horned beetle *Ontophagus acuminatus* (Emlen, 1997), or other dung beetles (Moczek *et al.*, 2002; Macagno *et al.*, 2011), are exceptional in the animal kingdom and often reflect discrete male strategies in the sexual context (Taborsky & Brockmann, 2010). Since we found both male morphs everywhere in the field and in all genetic lines of all populations, the morphs are determined largely by condition-dependent environmental plasticity, a so-called polyphenism (Moczek *et al.*, 2002). Nevertheless, the threshold curve here differed between populations even when reared in a constant laboratory environment, suggesting some genetic basis subject to natural and sexual selection (Taborsky & Brockmann, 2010).

The sigmoid trade-off relationship exhibited a short-term plastic response to developmental temperature as well as long-term population differentiation, presumably an evolutionary response due

to local adaptation to the environmental surface temperature, but these two responses to temperature differed. While an increase in developmental temperature shifted the curve slightly leftwards (Fig. 4a; parameter *e* in Table 1), higher environmental surface temperature primarily strongly augmented melanisation of the amber morph (upward shift in Fig. 4b; parameter *c* in Table 1). Increased developmental temperature is expected to shift the curve leftwards by reducing the body size of the flies according to the temperature-size-rule (Atkinson, 1994; Fig. 1), as also observed in the related *S. punctum* (Berger *et al.*, 2013), if only as a minimum baseline because unnatural constant temperatures were used in the laboratory. Alternatively, the shift in the curve Fig. 4a could be due to developmental temperature influencing the melanisation rate (Gibert *et al.*, 1996; Solensky & Larkin, 2003), e.g. by reducing melanin production in *S. thoracica* males of a certain body size range. Either mechanism, or both combined, would shift the curve leftward with increasing temperature, thus especially reducing melanism in flies of intermediate body sizes. Such reduction in melanism decreases the absorption of radiation (Hackman, 1953; Murkin & Arango, 2009), causing lower body temperatures (Watt, 1969; Lindstedt *et al.*, 2009). By this strategy individuals could avoid overheating. Nevertheless, an organism's body temperature depends not only on its colour, but also on its body size (Digby, 1955; Willmer & Unwin, 1981). Hence, the reduction of melanism at particular body sizes suggests a delicate balance between the two traits to influence body temperature.

In contrast to developmental temperature (Fig. 4a), when considering the habitat characteristics of the populations, an increase in environmental surface temperature results in a steep increase of melanism in amber males (Fig. 4b). This increase is counterintuitive because it would raise the insect's body temperature (Watt, 1969; Lindstedt *et al.*, 2009), potentially leading to overheating. However, this effect appears to be countered by the surface thermal radiation downwards (Fig. 4d; parameter *c* in Table 1). Thermal radiation represents the long-wave radiation emitted by the earth and its atmosphere, and plays an important role for *S. thoracica* because the dung pats where the flies feed and reproduce

absorb a large part of short-wave solar radiation due to their dark colouration, and emit long-wave radiation in return. Long-wave radiation absorbance is not influenced by melanism because it exceeds the pigment's absorbance spectrum (Hackman, 1953; Murkin & Arango, 2009), but it is affected by body size. Larger individuals have lower heat exchange rates due to their lower surface-to-volume ratio, and they reach a higher temperature excess, which is the difference between the body and the ambient air temperature (Digby, 1955; Willmer & Unwin, 1981). Consequently, larger amber individuals are more prone to overheating, and they may compensate this by lower melanism, reducing the absorption of short-wave radiation. This decrease in melanism in the larger (amber) males in response to increased long-wave radiation (downward shift in Fig. 4d) would thus cancel out the increase in melanism due to the environmental surface temperature seen in Fig. 4b (upward shift). These opposing effects suggest evolution of an equilibrated response to maintain low melanism in amber males, which has other advantages (e.g. in sexual selection: Busso, 2017).

Melanin absorbs short-wave radiation (Hackman, 1953; Murkin & Arango, 2009), and this so-called surface solar radiation downwards shifts the curve leftwards and reduces the steepness of its slope (Fig. 4c; parameters b & e in Table 1). The absorption of short-wave radiation results in increased body temperature (Watt, 1969; Lindstedt *et al.*, 2009), thus promoting overheating risk. Furthermore, melanin content also increases the rate of heat exchange of the individual (Brakefield & Willmer, 1985). Hence, the lower the melanism of an individual, the less it will have to be reduced to avoid overheating. The low melanism of the amber males probably already confers low to null absorbance of short-wave radiation, so no further melanism reduction is needed to avoid overheating. The small obsidians, in contrast, probably need to maintain high melanism to compensate for their high rates of heat loss due to their greater surface-to-volume ratio (Digby, 1955; Willmer & Unwin, 1981). Thus, absorbance crucially depends on body size, resulting in complex effects of short-wave radiation over the body size range of the flies.

323 Another insolation component that influenced our results was UV radiation (Fig. 4e; parameter
 324 b in Table 1). UV radiation has lethal effects on insects (Beard, 1972; Faruki *et al.*, 2007), as it directly
 325 damages DNA (Pfeifer, 1997) and/or enhances the production of reactive oxygen substances (Meng *et*
 326 *al.*, 2009; Sang *et al.*, 2012). Melanin protects cells against these harmful effects of UV radiation
 327 (Ortonne, 2002; Kvam & Dahle, 2003; Tada *et al.*, 2010), and this defensive role is also important in
 328 insects (Mosse & Lyakh, 1994; Hu *et al.*, 2013; Bastide *et al.*, 2014; Debecker *et al.*, 2015; but see Gunn,
 329 1998). Consequently, we would expect melanism to increase with increasing UV radiation for all
 330 individuals, but instead we observed an increase in the steepness of the slope (Fig. 4e; Table 1). This
 331 change in slope reinforces the subdivision of males into two morphs by minimizing the body size range
 332 for intermediate phenotypes, again suggesting that the morphs indeed could be using two different
 333 protection strategies against UV radiation. Obsidians invest in melanin, which protects them against UV
 334 radiation by directly absorbing it (Hackman, 1953; Murkin & Arango, 2009), whereas ambers
 335 alternatively invest more into body size. Since the thickness of insect cuticle increases with body size
 336 (Evans & Sanson, 2005), and UV radiation has very low penetration (Meinhardt *et al.*, 2008), it is possible
 337 that beyond a threshold body size (inflection point of the curve) the cuticle is thick enough to halt the
 338 penetration of UV radiation altogether. Melanin would then no longer be needed beyond this threshold
 339 and resources could be redirected into body size. This would result in a melanin - body size trade off, as
 340 reported in some other insects (Cotter *et al.*, 2008; Harris *et al.*, 2012). Alternatively, ambers could
 341 invest in a suite of protective macromolecules (heat shock proteins, cytochrome P450, or antioxidant
 342 enzymes) as safeguards against UV radiation (Meng *et al.*, 2009; Feyereisen, 2011). However, there is
 343 little evidence for a trade-off between melanin and these protective macromolecules in the literature.
 344 Future work should consequently address in detail the effect of UV radiation on the production of
 345 melanin and the thickness of the insect cuticle, as well as the role of protective macromolecules in both
 346 morphs to disentangle their effects. Of course, in the wild behavioural thermoregulation by individual

flies tailored to their phenotype (e.g. by avoiding unfavourable and/or actively seeking favourable microclimates around pastures and dung) must play a substantial and possibly paramount role in alleviating any of the detrimental effects of radiation discussed, and hence the selection pressures fostering these putative physiological and morphological adaptations.

All these environmental factors do not act separately, but rather simultaneously on the presented sigmoid relationship. Figure 5 shows the actual, integrated effects of the various factors as illustrated by the contrast between our northernmost (Pehka, Estonia) and southernmost *S. thoracica* populations (Adrano, southern Italy). The effects of environmental surface temperature and surface thermal radiation downwards (long-wave radiation; cf. Fig. 4b,d) on the melanism of amber males appear to cancel out each other, resulting in no melanism change. The increase in surface solar radiation downwards (short-wave), however, shifts the southern population leftwards, while the increase in downward UV radiation at the surface increases the steepness of its slope. These effects result in an overall, albeit slight increase in melanism with latitude across our 15 populations (Fig. 2), which concurs with what has been observed in other insects (reviewed by Clusella Trullas *et al.*, 2007). Season length did not significantly influence the trade-off curve (Table 1). The short development time of *S. thoracica* (ca. 12 d at room temperature) allows for multi-voltinism even in the northernmost populations, which dilutes and ultimately probably removes strong effects of seasonal time constraints on this species' life history (Blanckenhorn & Demont, 2004; Nygren *et al.*, 2008). These integrative influences of temperature and insolation seem to mostly affect the intermediate sections of the curve (Fig. 3), leaving the extreme morphs largely unaltered.

Both morphs were present in all isofemale lines, and all populations, indicating that the morphs are not genetically determined but rather present a plastic polyphenism (Moczek *et al.*, 2002). We showed that the sigmoid relationship between body size and melanism not only represents plasticity, but is also plastic itself. Developmental temperature slightly reshapes this relationship, adjusting the

male morphospace to the different thermal profiles. Hence, this plastic response can act as a buffer when facing new environmental conditions resulting e.g. from climate change (Chown *et al.*, 2007; Boggs, 2016; Sgrò *et al.*, 2016). At the same time the sigmoid relationship varied significantly among populations even when the environment was held constant, also indicating a genetic basis (Taborsky & Brockmann, 2010).

To conclude, melanism, but not body size, of *S. thoracica* increases clinally with latitude, an albeit slight effect with probably limited biological significance. Nevertheless, several climatic variables were shown to contribute considerably to geographic differentiation among populations that can be interpreted as adaptive (Figs. 4 & 5). The sigmoid trade-off between melanism and body size in male *S. thoracica* not only defines the plasticity of the male phenotypes, but is also plastic itself. It seems to evolve across populations in response to climatic factors such as temperature, insolation and UV radiation, which appear to have played complementary roles, and suggests that plasticity has an underlying genetic basis. As the plastic and evolutionary responses to temperature (and other factors) differed from one another, plasticity does not necessarily follow the direction of evolution of the trade-off, but rather adds to it (Fusco & Minelli, 2010). The environmental effects on body size and melanism demonstrated here will influence the fitness of *S. thoracica* individuals beyond their role in sexual selection (Andersson, 1994; e.g. Puniamoorthy *et al.*, 2012; Busso, 2017) or predation vulnerability (Berger *et al.*, 2006; Svanbäck & Eklöv, 2011; Busso, 2017). The correlative nature of our approach does not allow us to identify the causes of the evolutionary changes; nevertheless, our results suggest roles of some of the various climatic factors considered for the evolution of the body size - melanism trade-off in *S. thoracica* males. To our knowledge, this is the first study systematically addressing the influence of climatic factors on polyphenic sigmoid trade-offs, highlighting their possible role of for the evolution of polymorphisms.

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- 558

559 **DATA ACCESIBILITY:** We plan to upload all data to Dryad after acceptance of the paper.

560

561 **BIOSKETCH:** We are generally interested in the evolutionary ecology and short- and long-term evolution
562 of life history traits in various dung flies, including analysis of how climatic factors influence the allocation
563 and plasticity of fundamental phenotypic traits such as body size and colouration.

564

565 **AUTHOR CONTRIBUTIONS:** WUB and JPB conceived the project together. JPB performed the research,
566 analysed the data and wrote the manuscript, with significant input from WUB. The authors had some help
567 of collectors and technicians maintaining the flies in the laboratory.

568

569 **HANDLING EDITOR:** Simone Fattorini

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Table 1. Influence of various environmental habitat variables on the five parameters b – f defining the sigmoid logistic regression relationship between body size and melanisation of male *Sepsis thoracica* from Europe (see Methods for detailed explanation).

Environmental Variables	b (slope factor)	c (asymptote)	d (zero intercept)	e (mid-range value)	f (asymmetry)	Inflection Point	Minimum Slope
Developmental Temperature	-8821.633 ($\pm$ 73537.844)	-8.075 ($\pm$ 12.558)	23.252 ($\pm$ 14.954)	-0.003 ($\pm$ 0.001)***	0.97 ($\pm$ 0.031)	-0.002 ($\pm$ 0.001)***	-1.009 ($\pm$ 0.021)
Surface Solar Radiation Downwards	-3.833 ($\pm$ 1.453)**	-0.020 ($\pm$ 0.017)	0.119 ($\pm$ 0.063)	-0.192 ($\pm$ 0.061)*	0.013 ($\pm$ 0.034)	-0.090 ($\pm$ 0.069)	-17.962 ($\pm$ 27.718)
Surface Net Solar Radiation	-41.659 ($\pm$ 148.636)	0.024 ($\pm$ 0.02)	-1.286 ($\pm$ 4.352)	0.041 ($\pm$ 0.054)	1.757 ($\pm$ 4.204)	0.007 ($\pm$ 0.041)	-0.366 ($\pm$ 0.508)
Downward UV radiation at the surface	3.509 ($\pm$ 1.702)*	0.124 ($\pm$ 0.912)	-0.127 ($\pm$ 0.096)	0.152 ($\pm$ 0.086)	32.918 ($\pm$ 125.224)	0.078 ($\pm$ 0.09)	-0.156 ($\pm$ 0.343)
Surface thermal radiation downwards	-81.772 ($\pm$ 85.54)	-0.072 ($\pm$ 0.029)*	-3.547 ($\pm$ 4.813)	-0.013 ($\pm$ 0.008)	0.606 ($\pm$ 0.211)	-0.011 ($\pm$ 0.006)	-1.729 ($\pm$ 0.345)**
Environmental Surface Temperature	46.942 ($\pm$ 35.165)	0.070 ($\pm$ 0.033)*	-0.479 ($\pm$ 0.295)	0.014 ($\pm$ 0.010)	2.145 ($\pm$ 0.946)	0.013 ($\pm$ 0.020)	-0.65 ($\pm$ 0.170)
Season Length	-304.587 ($\pm$ 3687.266)	0.060 ($\pm$ 0.061)	0.208 ($\pm$ 0.227)	0.017 ($\pm$ 0.025)	1.187 ($\pm$ 1.287)	0.001 ($\pm$ 0.017)	-0.705 ($\pm$ 0.447)

The numbers represent changes in response to a one standard deviation unit increase of the environmental variable (see Fig. 4 for visual representation). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

572

FIGURE LEGENDS

Figure 1. Mean ($\pm$ SD) body size of three male morphs from 15 European *Sepsis thoracica* populations at three developmental temperatures.

Figure 2. Melanism of three male morphs from 15 European *Sepsis thoracica* populations as a function of latitude (separate regression lines with 95% confidence regions).

Figure 3. Sigmoid relationship between body size and melanisation in male *Sepsis thoracica* from Europe, with their respective frequency distributions. The pictures show the amber and obsidian male morphs, and the changes in melanism of the fore femur.

Figure 4. Effect of several environmental factors having significant influences on the sigmoid relationship between body size and melanism in European *Sepsis thoracica* males. The blue curve represents the changes in the mean sigmoid relationship (red curve) resulting from a one standard deviation increase in a) developmental temperature; b) environmental surface temperature; c) surface solar radiation downwards; d) surface thermal radiation downwards; e) downward UV radiation at the surface.

Figure 5. Actual differences in the sigmoid male body size – melanism relationship between the northernmost (Pehka, Estonia) and southernmost *Sepsis thoracica* populations (Adrano, southern Italy) sampled.

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592 three developmental temperatures.

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614 **Figure 5.** Actual differences in the sigmoid male body size – melanism relationship between the
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617