Original Article

Viability selection by invertebrate predators in the polyphenic black scavenger fly *Sepsis thoracica*

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Predation is a major factor influencing the fitness and life history of animals. Two key traits affecting prey survival are body size and coloration. *Sepsis thoracica* males display a sigmoid relationship between these 2 traits, defining a size threshold above which investment in melanin drastically drops, producing small melanic (black) or large amber morphs. In trying to understand the evolution of this rare dimorphism, we performed laboratory predation experiments to estimate the intensity of adult viability selection exerted by various arthropod predators (bugs, flies, and spiders) on male body size and coloration. Selection was performed against 2 different backgrounds mimicking the natural habitat (dung and grass) in which the camouflage and/or warning effect of the morphs should vary. Body size was mainly under positive selection (larger survived better), which overpowered selection on coloration and varied somewhat among predator species but not backgrounds. No disruptive selection was found, nor did selection change the sigmoid relationship between the 2 traits. We conclude that, for this fly, predator evasion and escaping skills determined by body size are more effective against invertebrate predators than its conspicuousness determined by coloration, contrasting what has been found for vertebrate predators, where prey coloration is important and negative selection on size dominates. Because arthropod predators have strong effects on insect populations, the positive directional selection imposed by invertebrate predators is likely an important force driving the evolution of body size in *S. thoracica* and insects in general.

Key words: **body size, coloration, Diptera, melanism, mortality, natural selection, polymorphism, predation, Sepsidae, threshold trait, trade-off, viability.**

INTRODUCTION

Predation is a major factor influencing the life history and morphology of species (reviewed by Ruxton et al. 2004). Avoiding predation involves numerous traits, and survival probability varies with each particular combination of defense traits as well as with the predator (Abrams 2000; Vamosi and Schluter 2004; Mikolajewski et al. 2006; Domenici et al. 2008). Two key traits drastically influencing the survival probability of prey are body size (Berger et al. 2006; Whitman and Vincent 2008) and coloration (Svanbäck and Eklöv 2011). There are both benefits and costs of being large in the predation context. For example, larger body size can increase the success of escaping an attack (Van Buskirk and Schmidt 2000; Whitman and Vincent 2008; Langerhans 2009). This advantage

mainly derives from larger sized prey having greater strength and better defenses against any particular handling skills of a given predator (Schmitt and Holbrook 1984; Shine 1991; Whitman and Vincent 2008). At the same time, larger body size augments the energetic value of the prey (Norberg 1978), making it more profitable for the predator (Gaston et al. 1997; Dixon and Hemptinne 2001). Moreover, larger size usually increases the detectability of prey (Karpestam et al. 2014).

Another important trait affecting prey survival is its coloration (Svanbäck and Eklöv 2011). In insects, a widespread strategy to intra-specifically alter coloration is through changes in melanin production (True 2003; Cook and Saccheri 2013), thus potentially increasing survival by decreasing prey detection and attack through crypsis or camouflage (Feltmate and Williams 1989; Johannesson and Ekendahl 2002; Fabricant and Herberstein 2015). Aposematism, by contrast, is a warning signal displayed by prey to signal its unpalatability to potential predators that makes individuals more conspicuous and ultimately reduces their likelihood of being attacked (Poulton 1890; Guilford 1990; Hargeby et al. 2004;

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see Ruxton et al. 2004 for further references and review). This warning effect can be enhanced by larger body size (Nilsson and Forsman 2003), hence coloration and body size should have combined effects on prey survival.

An excellent system to address how the interaction between body size and coloration influences predation is the black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae). Males of this species exhibit extreme variability in size and color, small males being mostly black (here called melanic) and the largest males bright orange (amber), whereas females are always completely black (Pont and Meier 2002). The black color of males is mediated by an increase in melanin investment that is largely environmentally induced (Schmid-Hempel 2003; Busso et al. 2017); it is hence a polyphenism that nonetheless has a heritable component (Busso and Blanckenhorn 2018a). Such threshold traits are generally rare and typically related to complex polymorphisms (Taborsky and Brockmann 2010), as observed, for example, in some dung beetles (Emlen 1997; Moczek et al. 2002). Male coloration could play a role in sexual selection for attracting mates (investigated elsewhere: Busso and Blanckenhorn, 2018c), attraction of predators being a concomitant negative consequence (Andersson 1994; Houde 1994). Alternatively, coloration could be directly aimed at predators, which was analyzed in the present study in an attempt to understand the evolution of *S. thoracica*'s male polyphenism.

As is typical, adult sepsid flies presumably face a wide range of generalist predators in their pastoral habitat, including vertebrates such as birds, amphibians, and lizards, but also many terrestrial invertebrates. Here, we performed laboratory viability selection experiments in 2 typical backgrounds using 3 representative predators of different arthropod guilds to investigate how body size and melanism affect *S. thoracica* predation and survival. We focused on invertebrate predators because they are more seldom studied but nevertheless may have stronger impact than vertebrates, especially on small insects (Fagan and Hurd 1994; Kristensen 1994; Wooster 1994; Lang et al. 1999). The size gap between *S. thoracica* and invertebrate predators is small relative to that with vertebrate predators; hence, we hypothesized that prey body size would influence survival primarily by affecting their evasion or escaping skills. We thus expected that survival probability would generally increase with prey size, but that there would be differences in selection intensity between the various arthropod predators due to their different size and hunting strategies. Alternatively, the detectability and energetic value of body size could play a greater role in predation, in which case survival probability should decrease with body size, varying among predators according to their differing energetic requirements and visual capabilities. Combined effects of prey evasion capability, detectability, and energetic value could result in an intermediate optimal prey body size for a given predator (Stephens and Krebs 1986).

We further considered the potential role of warning and camouflage for these invertebrate predators, even though we have no specific information on the spectral sensitivity of the sepsid visual system (cf. Supplementary Table S2). Since *S. thoracica* males search for females on and around cow dung pats (Pont and Meier 2002), we hypothesized that on the dung black color is cryptic, such that crypsis and survival probability would decrease as males become orange. Alternatively, the orange color could be a signal potentially warning predators of these flies' defense glands (Parker 1972a; Pont and Meier 2002), in which case orange males should survive better. In a colorful meadow, in contrast, orange might blend in better than black color, potentially reversing the situation.

We used standard statistical methods to estimate the intensity of viability selection on adult body size and melanism in *S. thoracica*, as well as any possible variation in selection intensity among predators (Lande and Arnold 1983; Arnold and Wade 1984). We studied selection in 2 natural backgrounds, dung, and grass, in which the significance of camouflage versus warning effect of fly color was expected to differ. Taking a bivariate approach permits disentangling the effects of prey body size and melanism on survival for the different predators and backgrounds; it also permits analysis of the effects of adult viability selection exerted by arthropod predators in altering the relationship between body size and melanism in this species.

MATERIALS AND METHODS

Sepsis thoracica maintenance and rearing

To represent the species in general, we worked with flies from 8 European *S. thoracica* populations along a latitudinal gradient: Ludwigshafen, Germany (49.48° N, 8.42° W); Nordrach, Germany (49.4° N, 8.08° W); Zürich, Switzerland (47.34° N, 8.54° W); Asturias, Spain (43.3° N, 6.0° E); Petroia, Italy (43.23° N, 12.56° W); Padula, Italy (40.34° N, 15.66° W); Lamezia, Italy (38.92° N, 16.25° W); and Adrano, Italy (37.67° N, 14.83° W). Wild-caught females were brought live to the laboratory to establish 10–20 replicate iso-female line cultures (i.e. the offspring of one field-caught female) per population. These fly families were maintained in separate plastic containers at 18 ± 1 °C and 14:10 h light:dark cycle, and regularly supplied with fresh cow dung, sugar, and water ad libitum.

To generate prey individuals, a dish with more or less dung, as occurs naturally, was placed for 24 h inside any of the *S. thoracica* iso-female line cultures for females to deposit eggs. This dung was incubated afterwards in a new container to obtain the adult males to be used in our predation trials, which crucially varied in size but otherwise showed natural behavior. Flies used in our experiment had been in the laboratory between 12 and 24 generations (ca. 1 month per generation). In total, we used 757 flies of total body lengths ranging between 4.87 and 9.14 mm and body weights between 0.4 and 2.4 mg.

Predator collection and maintenance

We sampled the arthropod community in grassland and forest edges around our university campus using sweep nets to collect predators that co-occur with the sepsids. Each predator was placed alone in a container with several *S. thoracica* individuals to identify taxa that feed on *S. thoracica*. Of those, we selected the species with the highest abundances in the sampled environments. We ultimately selected 3 visually oriented predators from 3 different guilds and of different body sizes so that the prey's coloration and background likely play a role for prey survival (Parker 1965, 1969; Land 1974; Harland and Jackson 2000; Teuschl et al. 2010): the jumping spider *Heliophanus flavipes* (Araenea: Salticidae), an active hunter between 6–9 mm long (Nentwig et al. 2016); the damsel bug *Himacerus mirmicoides* (Heteroptera: Nabidae), a sit-and-wait predator between 11–14 mm long with polyphagous feeding habits (Lattin 1989); and the predatory yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae), an active hunter between 6–12 mm long (Teuschl et al. 2010). All these predators are in principle large enough to subdue the prey offered and suck out the content of their prey leaving the exoskeleton behind, allowing us to measure the traits of the

captured prey after predation. To eliminate potential sex biases, we used only female predators, which as a rule should be more voracious.

The spider and bug predators collected in the field were maintained individually in plastic containers, provided with water in cotton-stoppered vials, and fed regularly with a mix of *Drosophila* species (*D. melanogaster*, *D. virilis*, *D. americana*, and *D. novamexicana*). Individuals were used in the predation experiments only after reaching their adult stage. All *S. stercoraria* individuals were raised in the laboratory.

Predation experiment

Prior to predation trials, each predator was kept in abstinence for 5 days, with water provided regularly. Right before starting the trials, the jumping spiders and the bugs were photographed dorsally under a stereo microscope MZ12 with a DFC490 camera (Leica). We measured the body size of the predator from digital photographs using ImageJ. For the spiders, we used the area of the cephalothorax as a proxy of body size; for the bugs, the area of their pronotum; and for *S. stercoraria*, hind tibia length.

We used as prey *S. thoracica* flies from random lines and populations to reduce any possible effect of local adaption to predators. Before each trial, male morphs were separated from each other and females under a stereo microscope MZ12 (Leica) using a transparent aspirator. The separated flies were placed in plastic containers and immediately transferred to the experimental arena. In each trial, we presented flies from only one randomly chosen iso-female line. The random use of lines from different populations reduced the possible effect of inbreeding on the experimental variables, as different iso-female lines would have randomly fixed different alleles.

The predation trials were carried out in a rectangular acrylic arena (10 (W) × 20 (L) × 10 (H) cm^3) provided with sugar and water ad libitum. We placed into the arena a randomly selected predator, plus 6 *S. thoracica* males (3 large ambers and 3 small melanics). Each trial was randomly assigned to one of the 2 backgrounds: dung or grass. The backgrounds consisted of a printed photograph of the natural habitat of the flies that covered the 4 sides of the arena and the bottom, leaving the top clear. The arenas were placed under 58W lamps emitting light in the visual and UV spectrum (FB58, Arcadia) to simulate natural lighting conditions. The experiments ran at 24 ± 1 °C and 16:8 h light:dark cycle. A trial concluded when 3 flies had been eaten by the predator, and we scored survival as a dichotomous variable (alive/dead). This temporal calibration of the experiment was necessary because the predators varied substantially in how fast they ate their prey depending on species, hunger level, etc., and because selection coefficients cannot be computed if none or all prey survived, half of them being optimal. As we controlled the presence of dead individuals during the day, there were only few cases where the number of dead individuals surpassed 3, but it was never 6. There were 21–23 replicates per predator-background treatment combination.

After the experiments, we measured body size and melanism of all preyed and surviving *S. thoracica* males. Since contents are sucked out by the predators, causing the shrinkage of the prey body, we used the foreleg femur, which does not shrink, for estimating both body size and melanism, based on preliminary analyses. (The size and coloration of all body parts are highly correlated in *S. thoracica*; see http://sepsidnet-rmbr.nus.edu.sg/ for pictures of many species.) Prey flies were dissected and their foreleg femur was photographed under a stereo microscope MZ12 with a DFC490 camera (Leica)

against a neutral white background. The camera was calibrated with a mini IT-8 calibration target to guarantee color consistency between the many pictures. The target also served as a scale to measure size. We used a self-written code in ImageJ, which measured the number of pixels of the foreleg femur and converted them to the real area according to the scale for each picture. The code also measured the number of melanic pixels in the femur, guaranteeing an objective quantification of melanism as the inverse of orange coloration. Any pixel with a V-value over 163 in the YUV color space was defined as melanic, corresponding to the valley in the bimodal V-value distribution of femur coloration (Y: brightness; U: blue-luminance; V: red-luminance; see https://en.wikipedia.org/wiki/YUV; cf. Figure 1, right; Busso and Blanckenhorn 2018a). Melanism represented the proportion of black and brown pixels over all the pixels of the femur.

Statistical analyses

We used standard regression methods to estimate uni- and bivariate linear and quadratic (nonlinear) selection coefficients (Arnold and Wade 1984; Lande and Arnold 1983) assessing the intensity of viability selection by predation on body size and melanism of male *S. thoracica* (see e.g. Blanckenhorn 2010, for a detailed textbook explanation). Univariate coefficients integrate direct and indirect selection on traits, while bivariate coefficients estimate the selection on one trait controlling for selection on another. The latter permits disentangling selection on multiple traits even when correlated, and also gives the resulting direction of selection acting on each trait (Lande and Arnold 1983; Arnold and Wade 1984). Linear coefficients indicate directional selection and quadratic coefficients disruptive (when positive) or stabilizing selection (when negative). Separately for each trial, we calculated standardized z-scores for body size and melanism of male *S. thoracica* by subtracting the trial mean from each value and dividing this difference by the standard deviation: $z_i = (x_i - \overline{x}) / SD_x$. Relative fitness w in each trial was calculated as the dichotomous absolute fitness (i.e. survival [1 or 0]) divided by the trial mean fitness given by the proportion of preyed flies (Arnold and Wade 1984). We used models of relative fitness on z-scored body size and melanism, $w = c + \beta_1 z$, to estimate univariate linear selection coefficients separately for each variable, where w is relative fitness, β_1 is the univariate linear selection coefficient, and z is the standardized trait, i.e. body size (bs) or melanism (m). c is the intercept in all models. To estimate bivariate linear selection, we used the model $w = c + \beta_{2bs} z_{bs} + \beta_{2m} z_m$, where β_{2bs} and β_{2m} are the bivariate linear selection coefficients of body size and melanism, respectively, and z_{bs} and z_m are the standardized body size and melanism. For the univariate quadratic selection, we used the model $w = c + \beta_1 z + \gamma_1 z^2$, where γ_1 is half the univariate quadratic selection coefficient; and for the bivariate quadratic selection differentials, we used the corresponding model $w = c + \beta_{2bs} z_{bs} + \beta_{2m} z_m + \gamma_{2bs} z_{bs}^2 + \gamma_{2m} z_m^2 + \gamma_{2bs \times m} z_{bs} z_m$, where γ_{2bs} and γ_{2m} are half the bivariate quadratic selection coefficients of body size and melanism, respectively, and $\gamma_{2bs \times m}$ is the correlational (i.e. interactive) selection between the 2 traits. As is standard in such selection studies, the linear terms of the bivariate models are not interpreted. As is also standard, statistical significance of all these estimates was tested using the corresponding full binomial (i.e. untransformed) models with absolute (i.e. binary: 1/0) survival as the outcome, predator species and background as fixed factors, prey body size and/or melanism as well as predator body size nested within predator species as continuous covariates, and trial as random effect, using the function "glmer" in the R package
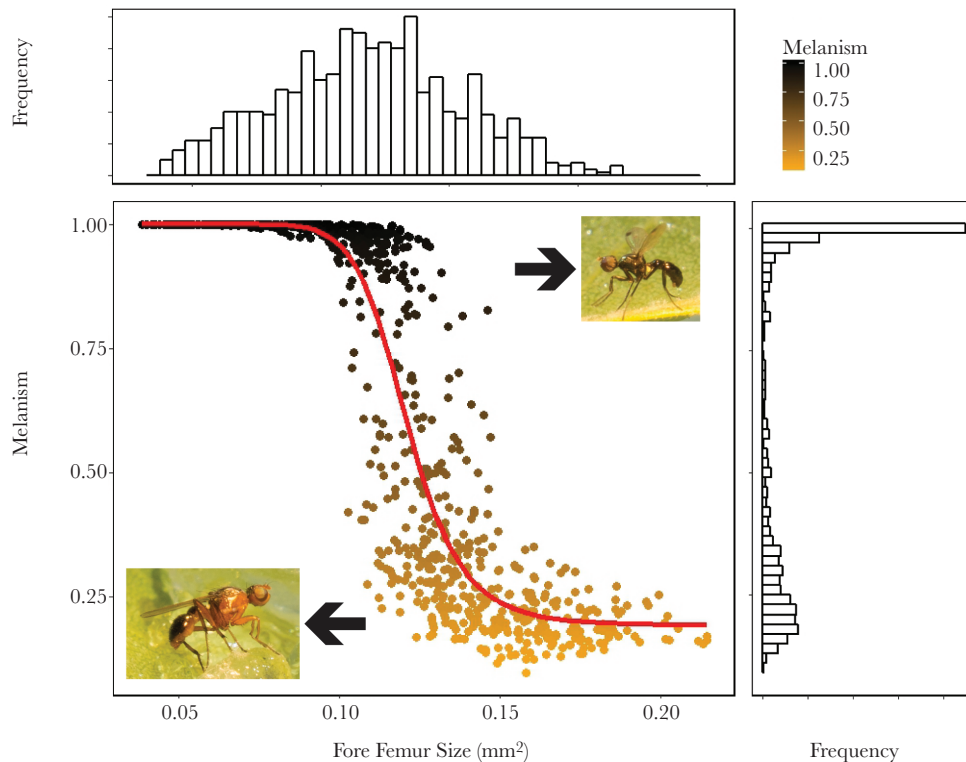
Figure 1
Relationship between body size and melanism for the *Sepsis thoracica* males used in our study, and their respective frequency distributions.

"lme4." All relevant (higher order) interactions were first included, though later removed from the final model if nonsignificant. We additionally partitioned the dataset by predator and background to calculate, for each treatment combination, all the selection coefficients mentioned above.

For the relation between body size and melanism in *S. thoracica* we fitted a 5-parameter logistic regression model: 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 at the inflection point, and f is the asymmetry factor (Gottschalk and Dunn 2005). To estimate the effects of selection on the sigmoid relationship between body size and coloration (selection differential), we compared the general 5-parameter logistic regression fitted to all *S. thoracica* individuals ($n = 757$; before selection) to another regression fitted just to the surviving individuals ($n = 360$; after selection). We evaluated statistical differences between all 5 parameters of both regressions using *t*-tests, with the means and standard deviations provided by the regression models (cf. Busso and Blanckenhorn 2018a). We also did the same for the subsample of flies in each background treatment. All analyses were performed using the software R Version 3.2.2 (R Development Core Team 2015).

RESULTS

Relationship between body size and melanism

We found a sigmoid relation between body size and coloration in *S. thoracica* across all 8 sampled populations, featuring a unimodal body size and a bimodal coloration distribution (Figure 1). This sigmoid relationship remained unaffected by the predation selection exerted here, as we found no significant changes in any

curve parameter before and after selection under any conditions (Supplementary Table 1).

Univariate selection on body size

Of the 757 flies used, 397 were eaten. The body size of the predators never had any significant effect on survival, either singly or in interactions with prey body size or melanism. We found overall positive directional selection for larger body size by invertebrate predation in *S. thoracica* in the full univariate model, i.e. when body size was considered alone (β_{1bs} in Table 1). In this model, we also found a significant 3-way interaction between prey body size, predator species, and background (Figure 2a and b, likelihood ratio $X_2^2 = 7.51$, $P = 0.023$). However, this was solely due to lacking selection by the damsel bug *H. mirmicoides* in a grass background (Figure 2a), as confirmed by removing this treatment from the dataset, which rendered the 3-way interaction nonsignificant (likelihood ratio $X_1^2 = 3.12$, $P = 0.108$), whereas positive directional selection remained significant and largely unchanged ($\beta_{1bs} = 0.417 \pm 0.106$, $P < 0.001$; cf. Table 1). Crucially, the 2-way interaction between body size and background was not significant (likelihood ratio $X_1^2 = 0.53$, $P = 0.463$), nor was the interaction between body size and predator species (likelihood ratio $X_2^2 = 2.63$, $P = 0.269$), indicating no variation in selection among predators or backgrounds.

Univariate selection on melanism

Melanism showed negative directional selection (against the melanic individuals) in the univariate model (β_{1c} in Table 1), as well as a slight but significant interaction between predator species and background (Figure 2c and d, likelihood ratio $X_2^2 = 8.25$, $P = 0.016$). However, the 2-way interactions between melanism and background (likelihood ratio $X_1^2 = 1.36$, $P = 0.243$) and between

Table 1

Selection coefficients ± SE for body size and melanism (i.e. the inverse of orange coloration; $n = 757$)

Predator	Background	Body size				Melanism				Interaction
		$\beta 1_{bs}$	$\beta 2_{bs}$	$\gamma 1_{bs}$	$\gamma 2_{bs}$	$\beta 1_{m}$	$\beta 2_{m}$	$\gamma 1_{m}$	$\gamma 2_{m}$	$\gamma 2_{bs \times m}$
All	Both	**0.417*** (±0.108)**	**0.816*** (±0.244)**	0.056 (±0.316)	−0.020 (±0.399)	**−0.354*** (±0.108)**	**0.422* (±0.232)**	0.113 (±0.317)	0.165 (±0.396)	0.104 (±0.260)
H. flavipes	Dung	**0.417*** (±0.116)**	**0.733* (±0.341)**	0.056 (±0.340)	0.736 (±1.282)	**−0.354*** (±0.117)**	**0.348* (±0.347)**	0.114 (±0.346)	0.368 (±0.641)	0.712 (±1.224)
	Grass	**0.312*** (±0.091)**	**0.719*** (±0.215)**	0.056 (±0.264)	0.678 (±0.998)	**−0.206* (±0.093)**	**0.454* (±0.215)**	0.007 (±0.260)	0.339 (±0.499)	0.642 (±0.838)
H. mirmicoides	Dung	**0.296*** (±0.100)**	0.245 (±0.238)	0.128 (±0.270)	−0.228 (±0.694)	**−0.279** (±0.101)**	0.061 (±0.257)	0.373 (±0.245)	−0.114 (±0.347)	−0.203 (±0.663)
	Grass	−0.052 (±0.111)	−0.045 (±0.231)	0.396 (±0.296)	0.362 (±0.576)	0.047 (±0.111)	0.088 (±0.235)	0.345 (±0.195)	0.181 (±0.288)	0.174 (±0.487)
S. stercoraria	Dung	**0.219* (±0.109)**	0.133 (±0.232)	0.280 (±0.264)	0.368 (±0.660)	0.098 (±0.232)	−0.055 (±0.237)	0.188 (±0.207)	0.184 (±0.330)	0.176 (±0.492)
	Grass	**0.529*** (±0.112)**	0.28 (±0.255)	0.156 (±0.318)	0.018 (±0.840)	−0.277 (±0.255)	−0.281 (±0.264)	−0.348 (±0.308)	−0.009 (±0.420)	−0.252 (±0.736)

Univariate ($\beta 1$) and bivariate ($\beta 2$) linear selection coefficient; univariate ($\gamma 1$) and bivariate ($\gamma 2$) quadratic selection coefficient; $\gamma 2_{bs \times m}$: correlational (interactive) selection coefficient. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

melanism and predator species (likelihood ratio $X_2^2 = 1.22$, $P = 0.544$) were not significant, again indicating no variation in selection among predators or backgrounds.

Bivariate selection on body size and melanism

In the bivariate model, both body size and melanism became significant (Table 1), melanism now being positively selected, a reversal in direction from the univariate model (compare β_{2c} and β_{1c} in Table 1), whereas the positive selection on body size remained. The 3-way interaction between body size, predator species, and background again was also significant (likelihood ratio $X_2^2 = 7.13$, $P = 0.028$), as was the interaction between melanism and predator species (likelihood ratio $X_2^2 = 6.74$, $P = 0.034$). However, the 3-way interaction between melanism, predator species, and background was not significant (likelihood ratio $X_2^2 = 0.203$, $P = 0.890$), nor was the interaction between melanism and background (likelihood ratio $X_1^2 = 0.007$, $P = 0.934$). Quadratic selection coefficients were never significant, nor were any of their interactions with the factors (Table 1). Importantly also, the correlational (i.e. interactive) selection coefficient was also not significant (Table 1, last column), suggesting independence of size and melanism despite their tight phenotypic relationship (Figure 1).

DISCUSSION

Sepsis thoracica males display a characteristic sigmoid relationship between body size and coloration (Figure 1), defining a body size threshold that subdivides males into a small melanic or a large amber morph, the evolution of which we are trying to explain by investigating current selection on both traits. Here, we found a positive relationship between the body size of male *S. thoracica* prey and their survival in laboratory predation experiments, documenting positive directional selection favoring larger body size by all the 3 arthropod predators used (Table 1). Although in the bivariate model controlling for body size amber individuals were preferentially preyed upon by at least one predator (the jumping spider *H. flavipes*) and overall (Table 1; Figure 2; resulting in significantly positive β_{2m} because melanic flies survived better), the corresponding univariate selection coefficients β_{1m} were mostly significantly negative, implying that (the larger) amber males were preyed upon less. In combination with rather weak and nonsignificant correlational selection and total lack of nonlinear disruptive or stabilizing selection (γ), the distribution of uni- and bi-variate directional selection coefficients (β) in Table 1 indicates that present viability selection by invertebrate predators mainly and more strongly targets body size rather than coloration (melanism). Likely for the same reason, the sigmoid relationship between the 2 traits (Figure 1) was not significantly altered by the predation selection documented here. Contrary to our a priori expectations about presumed roles of crypsis and/or warning effects of male coloration, the selection background (meadow vs. dung) turned out to be negligible. The greater influence of body size than coloration concurs with what has been observed for folivorous insect larvae facing various invertebrate predators (Dyer and Floyd 1993; Dyer 1997; Remmel and Tammaru 2009; Remmel et al. 2011). It is likely that in *S. thoracica* predator evasion and escaping skills determined by body size are more effective against invertebrate predators than the fly's conspicuousness or crypsis determined by its color.

Although of different guilds, all 3 predators exerted positive selection on prey body size in both backgrounds. This increase in survival with body size is likely mediated primarily by better
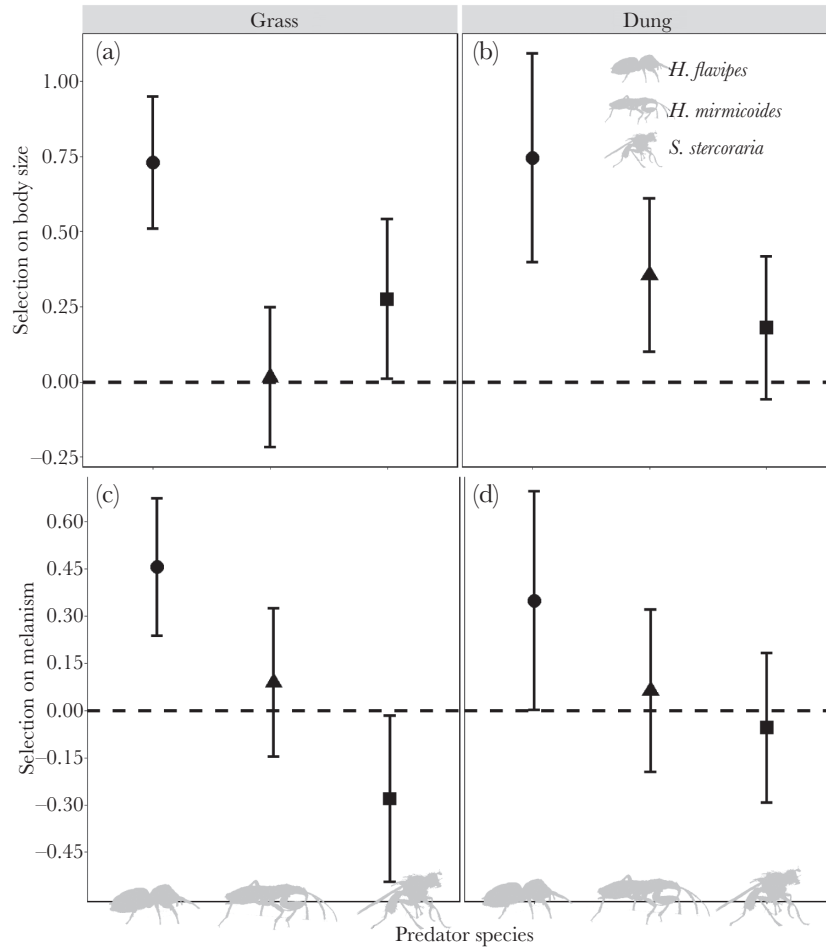
Figure 2
Mean (± SE) bivariate selection coefficients for body size (a and b) and melanism (c and d) for each predator in the dung and grass backgrounds. Positive values indicate selection for larger or darker individuals, while negative values indicate selection for smaller or more orange individuals, respectively.

evasion skills of the prey, as strength and size typically increase in parallel, and not so much by the energetic value of the prey for the predator (Wilson 1975; Griffiths 1980; Vermeij 1982; Bailey 1986; New 1992; Mänd et al. 2007; Okuyama 2007). These results concur with the reported positive size selection by *Scathophaga stercoraria* on the closely related *Sepsis cynipsea* (Teuschl et al. 2010), for which mobility differences did not explain size-dependent predation. Positive selection on body size has also been observed in the few studies addressing this issue with other invertebrate predators (Ovadia and Schmitz 2002; Whitman and Vincent 2008), suggesting that large prey size is of general advantage when facing invertebrate predators that are not much larger than their prey.

We observed positive selection on *S. thoracica* body size for all predators against all backgrounds, except in one scenario: the damsel bug *H. mirmicoides* in a grass background, where size selection was absent (Figure 2a). In this treatment, the melanic morph survived as well as the amber morph. Although vision has been shown to play an important role in the predatory behavior of some closely related bugs (Parker 1965, 1969), *H. mirmicoides* overall shows the weakest selectivity of all predators used (Table 1). It also could be that the damsel bug adjusts its feeding habits to the environmental background (Lattin 1989); or it could be that the predator evasion strategies used by the larger, amber *S. thoracica* males in the grass background are not effective against *H. mirmicoides*, whereas

those of the melanics are. Regardless, this outcome appears exceptional given our overall results, so we are well advised not to over-interpret it.

The bivariate selection coefficient for melanism (β_{2m}) was positive, whereas the univariate coefficient (β_{1m}) was negative. The latter means that small melanic males were disproportionately preyed upon by all predators, in accordance with the results for body size and the coupling of the 2 traits (Figure 1), such that large amber males showed higher survival (positive β_{1bs} and β_{2bs} in Table 1). However, when controlling for effects of body size, a preference for (large) amber males was apparent at least in the spider *H. flavipes*. Nevertheless, the effect of body size on predation success was overall much stronger than that of coloration / melanism, resulting in net selection against (small) melanic males (Table 1; Figure 2). Predation selection against small males, when combined with selection against (large) amber-colored males, does therefore apparently not exert strong enough disruptive selection and so does not disrupt the tight, perhaps even genetic correlation between color and size in *S. thoracica* males, also because we here observed no effect of selection on the shape of the relationship depicted in Figure 1. On the other hand, correlational selection ($\gamma_{2bs\times m}$), expected to be negative here, was also not found to be significant so as to strongly reinforce the coupling of the 2 traits (Table 1; Figure 1), in stark contrast to the situation for sexual selection (Busso and Blanckenhorn, 2018c).

The 2-way interaction between melanism and predator species was significant in the bivariate model, and the interaction between predator species and background was significant in the univariate model, suggesting that predation selection on melanism depended somewhat on the predator, but not the background in which this encounter took place. Nevertheless, the predators performed slightly differently in the 2 backgrounds (Figure 2c and d): particularly the yellow dung fly preyed more on the melanic morph (negative selection on melanism), while the bug showed no preferences, and the jumping spiders preyed more on the amber morph (positive selection). Selection on melanism was overall weaker in the dung background (Figure 2d), suggesting some background effect. Spectral sensitivity dissimilarities between the predator species could explain these differences (cf. Supplementary Table S2), although we think the evidence here is too weak to dwell on these. We rather conclude that motion is more important than coloration for prey detection by the predators used, as also reported for other invertebrate predators (Parker 1965, 1969; Land 1974, 1985; Wheater 1989). Sepsids are well known for their conspicuous, almost permanent wing-waving behavior facilitating their detection and defining their German name (*Schwingfliege*; Pont and Meier 2002), the function of which however is yet unclear. Some quantitative differences between the morphs were uncovered in their mating behavior (Busso and Blanckenhorn, 2018c), whereas behavioral differences were not obvious but unfortunately not assessed in the predation context here.

The positive selection on body size found here contrasts with the often found negative size selection exerted by larger vertebrate predators (Curio 2012; Remmel et al. 2011; Schülert and Dicke 2002; Tinbergen 1960). The most plausible reason is that the size gap between vertebrate predators and invertebrate prey is typically substantial, such that conspicuousness and detectability thresholds of predators likely result in preferences for larger prey individuals (Maiorana 1981; Winfield and Townsend 1983; Schülert and Dicke 2002; Shine and Thomas 2005; Troost et al. 2008; Curio 2012). In addition, vertebrate predators such as birds, lizards, or amphibians tend to be more sensitive to the higher energetic value of larger prey, again leading to preferences for larger prey items (Tinbergen 1960; O'Brien et al. 1976; Morin 1984; Stephens and Krebs 1986; Schülert and Dicke 2002; Curio 2012). The secondary role of coloration / melanism found here for invertebrate predators also contrasts with the results for vertebrates, which exert strong selection on prey color (Marples et al. 2005; reviewed by Ruxton et al. 2004). This contrast may be due to differences in the visual system in combination with the prey detection mechanism used. Most birds have sharp visual acuity and color vision encompassing a wide range of the color spectrum plus ultraviolet frequencies (Osorio and Vorobyev 2008), and often select their prey based on its coloration (reviewed by Stevens 2007). In contrast, the visual system of most insects and spiders is more constrained, being mono-, di-, or at best UV/blue/green trichromatic (Briscoe and Chittka 2001), such that detection of movement becomes more important (Parker 1965, 1969; Land 1974, 1985; Wheater 1989; Supplementary Table S2; Supplementary Figure S1). These differences between vertebrate and invertebrate predators highlight that the mechanisms employed, selective environments exerted, and evolutionary consequences of predators of the 2 taxonomic groups are not the same.

To conclude, here we investigated the influence of viability selection by invertebrate predators of different guilds in potentially shaping the sigmoid (threshold) relationship between body size and melanism in *S. thoracica* (Figure 1), ultimately trying to explain its evolution.

Although body size was under strong positive and melanism under negative selection (Table 1), we conclude that these selection forces alone cannot explain the size/coloration polyphenism of *S. thoracica* males. We had expected some sort of disruptive selection on coloration (Bond and Kamil 2002; Masta and Maddison 2002), but in all our models the quadratic (nonlinear) components were never significant. Alternatively, the plastic polyphenism might be mediated by disruptive selection on the underlying factor body size (Chevin and Lande 2013), but this was also not evident. Moreover, viability selection by invertebrate predators ultimately had no effect on the sigmoid relationship between body size and melanism because the sigmoid curves before and after selection were identical (Supplementary Table 1). Instead, we found that body size is generally under positive adult viability selection by various invertebrate predators, which all caught more small, melanic *S. thoracica* males throughout. Generally, positive selection on body size by invertebrate predators, with a secondary role of selection on coloration, contrasts with what has been observed for many vertebrate predators predominantly featuring negative selection on body size and strong selection on coloration. Nevertheless, the predation pressure by arthropod predators can be substantially greater than that exerted by vertebrates (Loiselle and Farji-Brener 2002), and several studies have shown that arthropod predators can have strong effects on insect populations (Fagan and Hurd 1994; Kristensen 1994; Wooster 1994; Lang et al. 1999). Consequently, the positive directional selection imposed by arthropods documented here is likely an important selective force driving the evolution of body size in invertebrates. However, given the multitude of predators of any given prey species, vertebrate or invertebrate, the corresponding net adult viability selection resulting in the wild can only be assessed comprehensively in a phenomenological way (when taking the prey perspective; e.g. Kalinkat et al. 2013), but not in a more mechanistic or functional manner as attempted here. To explain the evolution and maintenance of the tight threshold relationship between size and coloration in male *S. thoracica*, other selection forces, such as sexual selection, need to be considered (Busso and Blanckenhorn, 2018c).

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