




Does thermal plasticity align with local adaptation? An interspecific comparison of wing morphology in sepsid flies

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Abstract

Although genetic and plastic responses are sometimes considered as unrelated processes, their phenotypic effects may often align because genetic adaptation is expected to mirror phenotypic plasticity if adaptive, but run counter to it when maladaptive. Because the magnitude and direction of this alignment has further consequences for both the tempo and mode of adaptation, they are relevant for predicting an organisms' reaction to environmental change. To better understand the interplay between phenotypic plasticity and genetic change in mediating adaptive phenotypic variation to climate variability, we here quantified genetic latitudinal variation and thermal plasticity in wing loading and wing shape in two closely related and widespread sepsid flies. Common garden rearing of 16 geographical populations reared across multiple temperatures revealed that wing loading decreases with latitude in both species. This pattern could be driven by selection for increased dispersal capacity in the cold. However, although allometry, sexual dimorphism, thermal plasticity and latitudinal differentiation in wing shape all show similar patterns in the two species, the relationship between the plastic and genetic responses differed between them. Although latitudinal differentiation (south to north) mirrored thermal plasticity (hot to cold) in *Sepsis punctum*, there was no relationship in *Sepsis fulgens*. While this suggests that thermal plasticity may have helped to mediate local adaptation in *S. punctum*, it also demonstrates that genetic wing shape differentiation and its relation to thermal plasticity may be complex and idiosyncratic, even among ecologically similar and closely related species. Hence, genetic responses can, but do not necessarily, align with phenotypic plasticity induced by changing environmental selection pressures.

KEYWORDS

adaptation, climate change, clines, cogradients, countergradients, dispersal, genetic accommodation, genetic differentiation, geometric morphometrics, latitude, phenotypic plasticity

1 | INTRODUCTION

Temperature causes predictable plastic responses and may prompt the evolution of life history, morphology, behaviour

and even genetic architecture, throughout the tree of life (Alho et al., 2010; Allen, 1877; Atkinson, 1994; Atkinson, Morley, & Hughes, 2006; Berger, Stangberg, & Walters, 2018; Bergmann, 1847; Clauss, Dittmann Marie, Müller Dennis, Meloro, & Codron, 2013; Ray, 1960; Schilthuizen & Kellermann, 2014; Taylor, Price,

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Skeats, & Wedell, 2016; Zaidi et al., 2017). This is mostly due to the temperature dependence of biochemical processes inherent to all biological systems, which has direct consequences for an individual's fitness (Berger et al., 2018; de Jong & van der Have, 2009; Hochachka & Somero, 2014). Given its profound effect, and ongoing global climate change, the great interest in understanding how organisms adapt to temperature, both in the short and in the long term, is not surprising. In this regard, patterns of thermal plasticity and genetic differentiation along latitude have received particular attention (Kelly, 2019; Phillimore, Hadfield, Jones, & Smithers, 2010; Schilthuizen & Kellermann, 2014; Stoks, Geerts, & De Meester, 2014).

In this context, plastic and genetic shape changes have been particularly scrutinized in insect wings. In most species, higher temperatures lead to reduced body size (temperature-size rule) and show associated plastic responses in growth and development time (Atkinson, 1994; de Jong & van der Have, 2009). Temperature can also drive the evolution of adaptive genetic differentiation. Examples include the formation of latitudinal or altitudinal clines in development times and voltinism (Zeuss, Brunzel, & Brandl, 2017), melanization (Karl, Geister, & Fischer, 2009) or hibernation behaviour (Demont & Blanckenhorn, 2008). These responses are often thought to represent alternative solutions and adaptive routes along thermal gradients. If the costs of plasticity are high and gene flow between populations low, theory predicts that evolution will favour thermal specialists, resulting in genetic differentiation along latitude. Increased plasticity and generalist strategies, on the other hand, are expected to evolve if its costs are minor, environmental cues are reliable, and genetic constraints do not put an absolute limit on evolution (Via & Lande, 1985). That is, both plastic and genetic responses are two possible evolutionary routes to adaptation along temperature gradients.

Comparisons of plastic and genetic responses of the same trait, however, have frequently revealed evidence for covariation between the plastic response and genetic differentiation. Such pattern may for instance arise if plasticity produces unfavourable phenotypes across a latitudinal gradient. Stabilizing selection may then canalize the phenotype via compensatory genetic adaptation, leading to *countergradient* variation (Conover & Schultz, 1995; sometimes also called "genetic compensation": Grether, 2005). An opposite pattern is expected if the plastic response is not strong enough (or constrained) to produce an optimal trait value at any given latitude. In such cases genetic differentiation may enhance and reinforce the plastic response, allowing populations to reach their fitness optimum, and hence establish *cogradients* (Conover, Duffy, & Hice, 2009; Conover & Schultz, 1995; Falconer, 1990; Price, Qvarnstrom, & Irwin, 2003). Similar patterns are expected under genetic accommodation (or assimilation) where plasticity precedes and biases subsequent genetic adaptation (Lande, 2009; Moczek, 2007; West-Eberhard, 2003).

Co- as well as countergradient variation along latitude is frequently found in insects (e.g. Berger, Bauerfeind, Blanckenhorn, & Schäfer, 2011; Blanckenhorn & Demont, 2004; Conover et al., 2009;

Kivela, Valimaki, & Maenpaa, 2012; Meister, Esperk, Valimaki, & Tammaru, 2017), and studying their evolutionary causes is helpful in understanding how phenotypic variation arises, and hence for predicting adaptation to future climate changes. It has however been argued that linear traits, such as body size or development time, may not be well suited for comparing patterns of plasticity and genetic differentiation because they only vary along one dimension (Pitchers, Pool, & Dworkin, 2013). Accordingly, as linear traits can only increase or decrease with temperature or latitude, chances are high that plastic and genetic responses appear qualitatively similar even in the absence of a common underlying selection pressure. By contrast, studying traits with high dimensionality can avoid spurious qualitative concordance between genetic differentiation and plasticity while still permitting quantitative comparisons, provided that the various traits under scrutiny are not strongly genetically correlated (and hence can vary and evolve as independent units; Walsh & Blows, 2009).

In this context, plastic and genetic shape variations of insect wings have been particularly scrutinized. Being the prime agent of dispersal in many pterygote species, wings are likely to be targets of natural selection (Gilchrist, Azevedo, Partridge, & O'Higgins, 2000). Although wings are doubtlessly important in foraging, predator avoidance and sexual selection, wing shape and size are also important for thermoregulation (Angilletta, 2009). In contrast to warm-blooded vertebrates, small-bodied insects have only limited capacity to regulate their body temperature intrinsically (Harrison, Woods, & Roberts, 2012). Hence, the majority of insects regulate their temperature by behavioural means (Chown, Chown, Nicolson, Nicolson, & Nicolson, 2004; Stevenson, 1985). Small, winged insects, such as drosophilids, therefore use flight to make best use of their heterogeneous habitats (Dillon, Wang, Garrity, & Huey, 2009). Nevertheless, the capacity for flight is severely hampered in the constant cold, and must be rescued by adjustments in wing shape and size (Dillon & Frazier, 2006; Frazier, Harrison, Kirkton, & Roberts, 2008; Stalker, 1980). Consequently, temperature-dependent plasticity as well as clinal genetic variation in wing loading (i.e. the ratio between body weight and wing area) or wing shape has been found repeatedly within and between species (Azevedo, James, McCabe, & Partridge, 1998; Fraimout et al., 2018; Gilchrist & Huey, 2004; Gilchrist, Huey, & Serra, 2001; Pitchers et al., 2013; Rohner et al., 2015, 2018; Schäfer et al., 2018; Stalker, 1980; Stalker & Carson, 1946; Starmer & Wolf, 1989).

Unsurprisingly, most of the literature on latitudinal clines and thermal plasticity in wing shape focusses on a few species of *Drosophila* (e.g. Imasheva, Bublil, Lazebny, & Zhivotovsky, 1995; Hoffmann & Shirriffs, 2002; Debat, Begin, Legout, & David, 2003; Fragata et al., 2010; Fraimout et al., 2018; Pitchers et al., 2013; Simões et al., 2015, but see: Thery, Gyulavári, Schillewaert, Bonte, & Stoks, 2014 and Schäfer et al., 2018). However, although drosophilids represent a powerful system that has greatly improved our understanding on how wing shape and size vary and evolve, it remains unclear to which extent the same patterns are also found in other species.

Revisiting two independent common garden rearing experiments by Berger, Postma, Blanckenhorn, and Walters (2013) and Roy, Blanckenhorn, and Rohner (2018), we here investigate the relationship between phenotypic plasticity and genetic differentiation in relative wing size and shape in two species of black scavenger flies (Diptera: Sepsidae). We quantify thermal plasticity and genetic latitudinal variation in wing shape and size, investigate to which extent these patterns are similar across species, and then ask whether the plastic and genetic responses are aligned. If genetic differentiation is adaptive, we expect convergent latitudinal clines in both species. Based on previous studies arguing that thermal plasticity in wing shape and size is adaptive, we further expect the plastic response to temperature to be aligned with the pattern of genetic differentiation along latitude, forming a cogradient in each species in which genetic adaptation adds to the effect of phenotypic plasticity.

2 | MATERIALS AND METHODS

Sepsis fulgens Meigen, 1826 and *Sepsis punctum* (Fabricius, 1794) are both common species of black scavenger flies (Diptera: Sepsidae) found throughout Central Europe (Ozerov, 2005; Pont & Meier, 2002). Being small, multivoltine acalyptrates developing in decaying organic substrates, sepsids are expected to be under similar ecological selection pressures as drosophilids, yet previous studies demonstrated contrasting latitudinal clines for life-history traits (also in the two species studied here: e.g. body size: Puniamoorthy, Schäfer, & Blanckenhorn, 2012; Roy et al., 2018). Although *S. punctum* has been argued to be adapted to warmer climates, these two rather closely related species can frequently be found in the same microhabitat, where both species preferentially use vertebrate dung as breeding substrate (mostly cattle dung or dung heaps; Pont & Meier, 2002; Rohner et al., 2015; Rohner & Bächli, 2016).

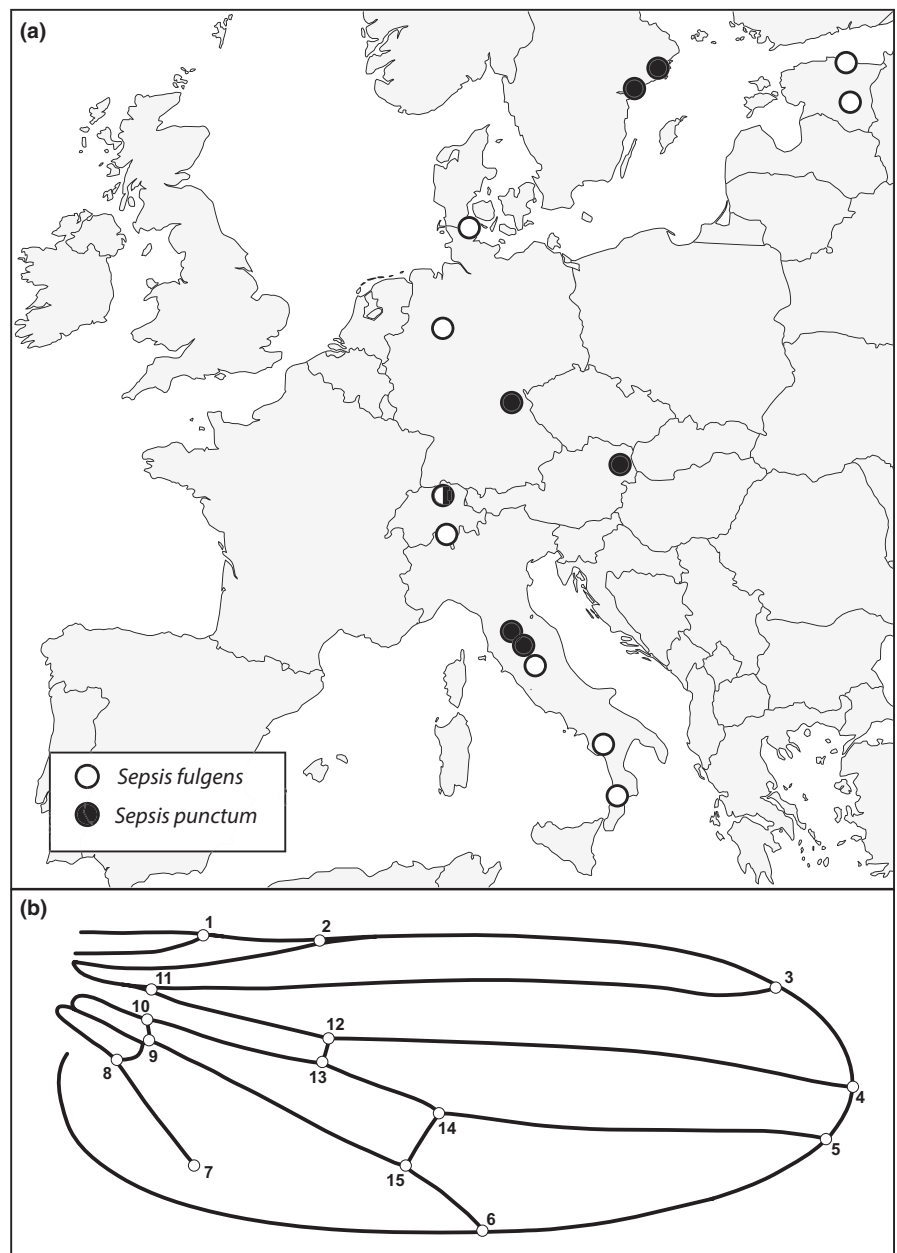


FIGURE 1 (a) Population sampling for *Sepsis fulgens* (open circles) and *Sepsis punctum* (filled circles) across Europe. (b) Shows the fifteen morphometric landmarks used to quantify genetic as well as plastic shape variation

Species	Population	Number of lines	Latitude (°)
<i>Sepsis fulgens</i>	Bielefeld, Germany	5	52.03
<i>Sepsis fulgens</i>	Lamezia, Italy	6	38.92
<i>Sepsis fulgens</i>	Padula, Italy	5	40.33
<i>Sepsis fulgens</i>	Pehka, Estonia	6	59.48
<i>Sepsis fulgens</i>	Rahinge, Estonia	5	58.37
<i>Sepsis fulgens</i>	Skelde, Denmark	3	54.85
<i>Sepsis fulgens</i>	Terni, Italy	5	42.57
<i>Sepsis fulgens</i>	Ticino, Switzerland	5	46.25
<i>Sepsis fulgens</i>	Zurich, Switzerland	6	47.34
<i>Sepsis punctum</i>	Arezzo, Italy	7	43.53
<i>Sepsis punctum</i>	Bayreuth, Germany	12	49.95
<i>Sepsis punctum</i>	Nyköping, Sweden	9	58.75
<i>Sepsis punctum</i>	Perugia, Italy	12	43.14
<i>Sepsis punctum</i>	Stockholm, Sweden	8	59.33
<i>Sepsis punctum</i>	Vienna, Austria	12	48.21
<i>Sepsis punctum</i>	Zurich, Switzerland	13	47.34

TABLE 1 Population origin, the corresponding latitude and the number of iso-female lines per population used in the common garden rearing for both species. Each line was reared in different temperature treatments (four temperatures in *Sepsis fulgens* and five temperatures in *Sepsis punctum*). Wing shape and thorax length were measured for two individuals per population, line, temperature and sex

Laboratory iso-female lines of both species were established using offspring of wild-caught, gravid females of several populations for *S. fulgens* (nine populations; Figure 1a, Table 1) or using the first filial generation of females emerging from individual dung traps deposited in the field (*S. punctum*: seven populations; Figure 1a, Table 1). Upon establishment, iso-female lines were provided with a continuous supply of water, sugar and fresh cow dung to be reared for several generations under laboratory conditions.

2.1 | Common garden rearing

To test for latitudinal (genetic) variation and thermal plasticity in (relative) wing size and shape, iso-female lines of all populations were reared under common garden conditions using several temperature treatments. Note that we here revisit a subset of animals that were reared in two temporally separated common garden settings used in previous studies (*S. punctum*: Berger et al., 2013; *S. fulgens*: Roy et al., 2018). These individuals were frozen with a drop of water to avoid evaporation and stored until recently dissected for morphometric measurements. In both common garden experiments, iso-female lines were provided with a small amount of homogenized, previously frozen cow dung for oviposition. After 24 hr, the dung was removed and eggs were retrieved. Larvae were then provided with standardized cow dung ad libitum, preventing larval competition for food and space and hence minimizing environmental effects. Berger et al. (2013) transferred individual first-instar larvae, whereas Roy et al. (2018) moved freshly hatched larvae with their substrate into new containers. Containers were then incubated immediately under different temperature regimes. Hence, individuals experienced their corresponding temperature treatments during their full larval (all three instars) and pupal

development. Upon emergence and complete hardening of the exoskeleton, adults were removed from the climate closets and killed immediately. In *S. fulgens*, offspring of each iso-female line were reared at four temperatures (12, 18, 24 and 30°C), whereas in *S. punctum*, five temperatures were used (15, 18, 23, 28 and 31°C). Note that although the temperature spectrum and the number of treatments do not correspond fully across species, patterns of quantitative genetic differentiation along latitude can still be compared when only considering individuals reared at 18°C because this temperature regime was applied in both species. Other effects of the two experimental blocks cannot be controlled for. This, however, should not affect our ability to compare morphological patterns of plasticity and differentiation within species.

2.2 | Morphometric measurements

For the morphometric analysis, we removed the right wing of two individuals per sex, iso-female line, temperature, population and species, and mounted it on a glass slide using Euparal. The dissected wing, as well as the thorax (lateral view), was photographed using a Leica DFC490 camera mounted on a Leica MZ12 microscope. The thorax was measured as the cumulative length of the scutum and the scutellum using digitized landmarks derived from tpsDig vers. 2.14 (Rohlf, 2009).

To quantify wing shape, we digitized 15 landmarks, again using tpsDig (see Figure 1b for a visual representation of the landmarks), which were used to calculate centroid size (a composite measure of overall wing size: Klingenberg (2016)), and retrieved Procrustes-transformed coordinates using the function *gpa* of the R-package *geomorph* (Adams & Otárola-Castillo, 2013). As an estimate of wing loading, we divided thorax length³ (i.e. volume) by the centroid size² (i.e. wing area).

2.3 | Statistical analysis

To assess latitudinal clines within species, we regressed the weighted average of thorax length and wing size per iso-female line against latitude using the population term as random effect (with the function *lme4* in R: Bates, Machler, Bolker, & Walker, 2015). To test for clinal variation in wing loading, we fitted wing size as a function of latitude (using the population effect as the error term) with thorax length as covariate. We here restricted our analyses to individuals raised at 18°C, permitting direct comparison between species (see above). Note that *S. punctum* and *S. fulgens* are closely related, but do not represent sister species (Rohner et al., 2014; Zhao, Ang, Amrita, Su, & Meier, 2013). Any clinal patterns are hence likely to be driven by convergent evolution and not due to shared ancestral variation.

To test for thermal plasticity, we again used linear mixed models based on line means per sex and temperature with population as random effect. As thermal reaction norms are usually nonlinear, we fitted sex-specific line means as a function of temperature, temperature², sex as well as the sex × temperature- and sex × temperature²- interactions. Clinal variation in plasticity was tested by adding latitude and the interactions with temperature and its squared term. Nonsignificant interaction terms were removed.

We tested for latitudinal variation in wing shape within species by using the multivariate regression approach implemented in *MorphoJ* (Klingenberg, 2011). We first averaged the Procrustes-transformed wing coordinates by population and regressed these averages against latitude. The statistical significance of these multivariate regressions was assessed using randomization tests (using 10,000 random samples). We used the same approach to test for allometric shape variation, thermal plasticity and sexual shape dimorphism but then used iso-female line means as the level of comparison.

To compare the latitudinal genetic differentiation in wing shape with the direction of the plastic response to temperature, we calculated correlations among their shape deformation vectors. To this end, we first applied a Bayesian multivariate general linear mixed-effects model utilizing Markov Chain Monte Carlo sampling (R-package *MCMCglmm*: Hadfield (2010)) to estimate the effects of latitude, temperature, size and sex on shape simultaneously (see: Schäfer et al., 2018). Because raw Procrustes-transformed coordinates are often prone to show high collinearity, we used their principal components (PCs) based on the covariance matrix for further analysis. PCs are by definition orthogonal and hence cause no computational issues related to multicollinearity. Because Procrustes superimposition results in a deficiency of four ranks, we only fitted the first 26 PCs (15 landmarks × 2 coordinates – 4 deficient ranks). *MCMCglmm*s were fitted separately for each species using iso-female lines and populations as random effects. The off-diagonal elements of the covariance matrix were set to zero (using the *idh* function of *MCMCglmm*) given the orthogonal structure of the PCs based on the total variation in the data. Uninformative flat priors were used for the residual and random effect covariance matrices. Models were run for 220,000

iterations using a thinning interval of 100, with the first 20,000 iterations being discarded (burn-in), resulting in 1,000 uncorrelated posterior estimates stored for further analysis. We estimated the magnitude of shape change in response to latitude, temperature, sex and size as the summed effect of all landmark movements (i.e. Procrustes distance).

To quantify the similarity between the effect of two variables on shape (e.g. thermal plasticity and genetic differentiation), we retrieved the vectors of shape deformations in Procrustes space v_1 and v_2 and computed the vector correlation between v_1 and v_2 as

$$r_{1,2} = \frac{|v_1 \cdot v_2|}{\|v_1\| \times \|v_2\|}.$$

That is, we scaled the dot product of v_1 and v_2 by their norm (c.f. Claude, 2008; Pitchers et al., 2013; Schäfer et al., 2018). This procedure was repeated for each stored posterior estimate of the *MCMCglmm* such that we could compute a 95% posterior density interval. We first assessed the similarity of the effects of size, rearing temperature, sex and latitude on shape between species and then tested for correlations among effects within species.

3 | RESULTS

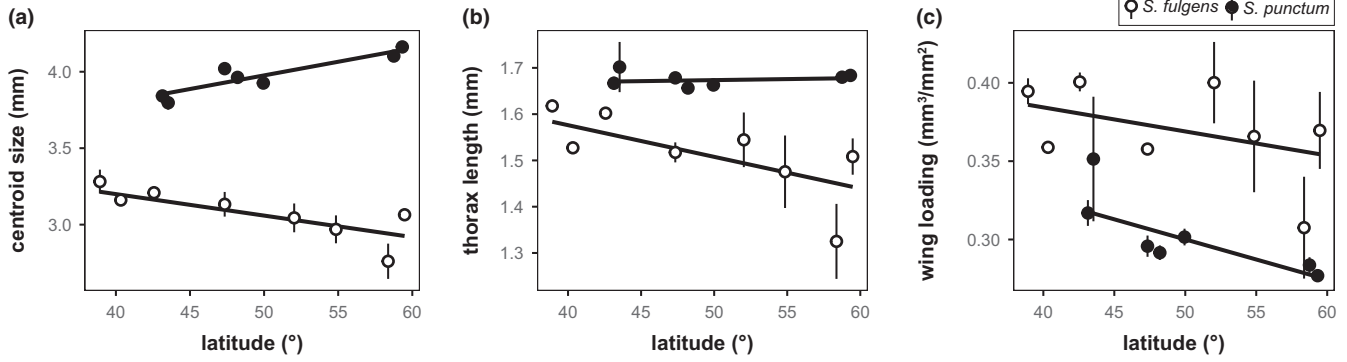
3.1 | Clinal variation and thermal plasticity in wing size and wing loading

Wing (centroid) size increased at higher latitude in *S. punctum* ($F_{1,5.29} = 25.48$, $p = 0.003$, Figure 2a), but decreased with latitude in *S. fulgens* ($F_{1,7.35} = 7.92$, $p = 0.025$, Figure 2a). Thorax length showed no latitudinal variation in *S. punctum* ($F_{1,5.1} = 0.19$, $p = 0.668$, Figure 2b), but a marginally nonsignificant decrease with latitude in *S. fulgens* ($F_{1,7.70} = 4.37$, $p = 0.071$, Figure 2b). Although the clines in wing and thorax size differed qualitatively between species, wing loading showed a consistent decrease towards the poles in both species, albeit marginally nonsignificantly so in *S. fulgens* (*S. punctum*: $F_{1,5.07} = 23.44$, $p = 0.005$, *S. fulgens*: $F_{1,7.93} = 4.56$, $p = 0.066$, Figure 2c).

Wing size, thorax length and wing loading showed nonlinear relationships with developmental temperature (temperature²: all $p < 0.001$; Table 2), a typical finding when studying thermal reaction norms (Angilletta, 2009). Thermal plasticity of wing size showed clinal variation in *S. punctum*, for which the nonlinearity of the thermal reaction norm steadily increased with latitude (latitude × temperature²- interaction: $F_{1,24} = 21.9$, $p > 0.001$; Figure 3). For all other traits, the latitude × temperature- and the latitude × temperature²- interactions were not significant (Table 2).

Both species were sexually dimorphic for all traits measured. Although males have larger wings, longer thoraces and lower wing loading in *S. punctum*, we found the opposite patterns in *S. fulgens* (Table 2), in which females are the larger sex (cf. Rohner, Blanckenhorn, & Puniamoorthy, 2016).

Clinal variation (18°C)



Thermal plasticity

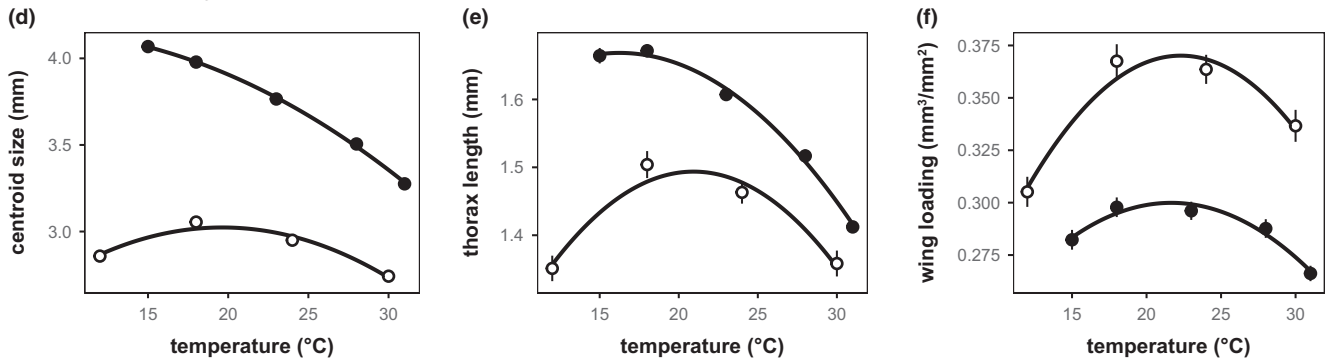


FIGURE 2 Clinal variation (a–c) and thermal plasticity (d–f) for wing centroid size, thorax length and wing loading for *Sepsis fulgens* (open circles) and *S. punctum* (filled dots). Although wing centroid size (a) and thorax length (b) clines differ qualitatively between the species, both species show a decrease in wing loading towards northern latitudes (c). Temperature effects on wing size (d), thorax length (e) and wing loading (f) were pronounced and nonlinear

TABLE 2 ANOVA tables (type III sums of squares). Thermal plasticity in wing centroid size, thorax length and wing loading show nonlinear reaction norms in both species. The sex × temperature²- interaction was not significant throughout and hence was removed. Analyses were based on iso-female line means with population origin as random effect in each analysis

	Wing centroid size				Thorax length				Wing loading			
	MS	ddf	F	p	MS	ddf	f	p	MS	ddf	F	p
<i>Sepsis punctum</i>												
Temperature	0.072	619.0	5.15	0.024	0.236	592.7	50.94	<0.001	0.162	591.0	186.52	<0.001
Temperature ²	0.856	619.0	61.59	<0.001	0.480	593.3	103.62	<0.001	0.045	591.3	51.92	<0.001
Sex	1.162	619.0	83.66	<0.001	1.205	591.4	260.43	<0.001	0.051	591.4	59.00	<0.001
Sex × temperature	0.884	619.0	63.60	<0.001	0.448	591.4	96.90	<0.001	0.037	591.0	42.04	<0.001
<i>Sepsis fulgens</i>												
Temperature	2.269	277.2	52.44	<0.001	1.050	278.3	58.17	<0.001	0.080	278.5	25.03	<0.001
Temperature ²	2.611	277.2	60.35	<0.001	1.060	278.3	58.74	<0.001	0.144	278.4	45.29	<0.001
Sex	0.764	277.2	17.66	<0.001	0.896	278.5	49.63	<0.001	0.128	278.4	40.29	<0.001
Sex × temperature	0.137	277.2	3.16	0.077				n.s.				n.s.

3.2 | Clinal variation and thermal plasticity in wing shape

We found significant allometric variation, thermal plasticity and sexual dimorphism for wing shape in both species (all $p \leq 0.014$). In contrast to *S. punctum*, which showed latitudinal variation in wing shape

($p = 0.043$, $n = 7$), *S. fulgens* did not show significant levels of genetic differentiation along latitude ($p > 0.5$, $n = 9$). When comparing vectors of model coefficients between species, that is the alignment of shape change observed in the two species in response to a particular explanatory variable, we found the effects of temperature on shape to correlate moderately but significantly between species ($r = 0.33$

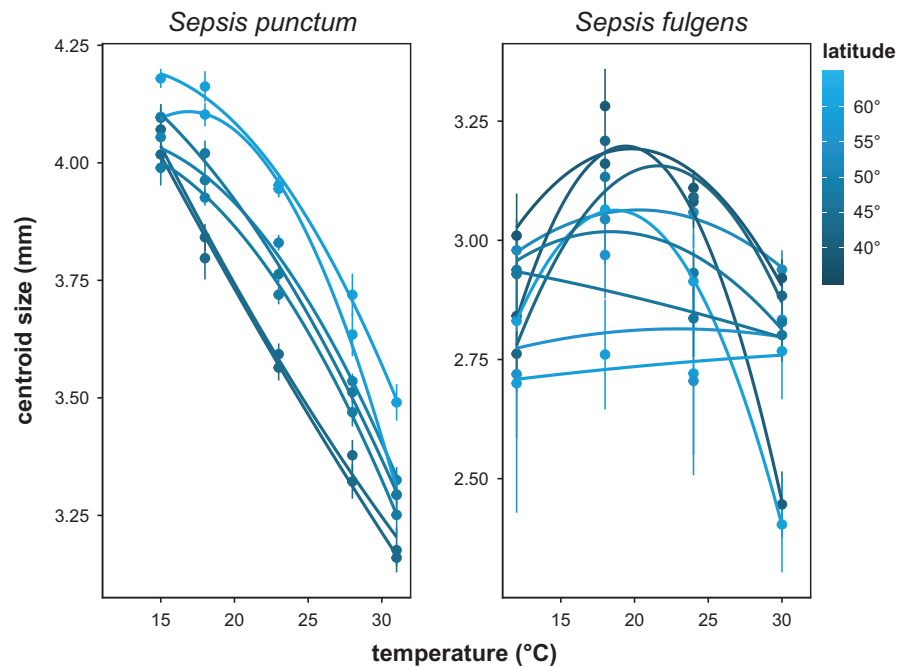


FIGURE 3 Clinal variation in phenotypic plasticity for wing centroid size in *Sepsis punctum* and *S. fulgens*. Although the former shows latitudinal population differentiation in thermal plasticity, the latter does not

[0.19, 0.46], Figure 4), although shape allometry ($r = 0.87$ [0.76, 0.92], Figure 4) and sexual dimorphism in shape ($r = 0.60$ [0.42, 0.92], Figure 4) were more conserved among the species. The genetic differentiation along latitude showed similar patterns in *S. punctum* and *S. fulgens*, but this correlation was not significantly different from zero ($r = 0.58$ [-0.07, 0.83], Figure 4), which is unsurprising given the lack of significant clinal variation in *S. fulgens* and the low statistical power associated with estimating these correlations (based on population rather than iso-female line means). *S. punctum* further responded more strongly to temperature, sex and latitude compared to *S. fulgens*, whereas the strength of the allometric effect did not differ significantly between species (Figure 4).

Thermal plasticity as well as sexual shape dimorphism was to some extent dependent on allometry in both species, whereas latitudinal differentiation was not (Figure 5). The latter result is unexpected, given that wing size shows clinal variation in both species. Interestingly, whereas the effect of latitude on shape was not correlated with the effect of temperature in *S. fulgens* ($r = 0.08$ [-0.47, 0.51], Figure 5), the two effects were significantly negatively correlated in *S. punctum* ($r = -0.59$ [-0.74, -0.37], Figure 5). As latitude is inversely related to temperature (colder at higher latitudes), the genetic latitudinal cline follows the pattern of thermal plasticity in wing shape within populations, that is northern populations show wing shapes similar to flies reared at cool temperatures. Given that the two species were not reared under identical conditions, species differences in thermal plasticity in wing shape could arise due to variation in the temperature range applied. Therefore, we repeated all analyses excluding temperatures below 18°C resulting in a very similar temperature range in both species (18–31°C vs. 18–30°C). The results remained qualitatively identical (except for an increase in the correlation between species; $r_{12-31^\circ\text{C}} = 0.33$ vs. $r_{18-31^\circ\text{C}} = 0.60$).

The negative correlation between temperature and latitude in *S. punctum* and its absence in *S. fulgens* remained, and hence, this species difference does not seem to depend on the chosen temperature range.

4 | DISCUSSION

We here studied the relationship between thermal plasticity and latitudinal differentiation in wing size and shape in two closely related dipterans. Wing loading showed strong (nonlinear) thermal plasticity as well as clinal variation in both species (decreasing towards higher latitudes). As our common garden rearing removed environmental variation, these clines are genetic, thus providing evidence of convergent evolution that suggests an adaptive response to selection for increased dispersal capacity in cold or more variable environments (c.f. Stalker, 1980; see below). The relationship between genetic differentiation and plasticity in wing loading is complex, however, as thermal reaction norms are nonlinear. Contrary to wing loading, wing shape clines and their association to thermal plasticity were inconsistent between species. In *S. punctum*, clinal genetic differentiation aligns with the effect of temperature, suggesting adaptive cogradient variation. In contrast, *S. fulgens* only showed minor (if any) latitudinal shape variation, which did not correspond to the plastic response. Genetic differentiation hence can—but does not necessarily—align with the effect of phenotypic plasticity. Our study illustrates the utility of comparing replicated genetic and plastic responses of complex phenotypes for understanding adaptive trait variation and evolutionary processes in populations adapting along latitudinal gradients. Below we discuss each of our results in greater detail.

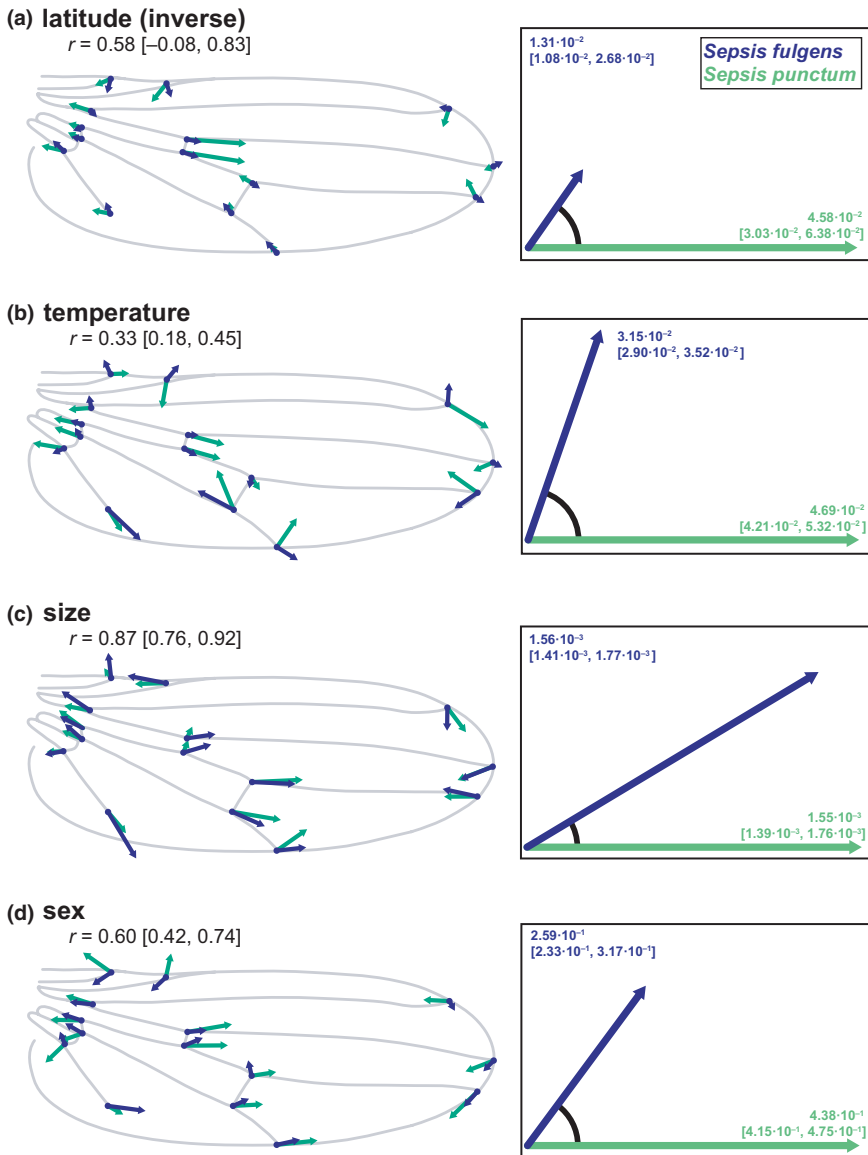


FIGURE 4 Changes in wing shape associated with latitude (a), temperature (b), size (c) and sex (d) for *S. punctum* (green) and *S. fulgens* (blue). Left: Arrows indicate the change in position of the respective landmark to an increase in a given explanatory variable. The overall correlations between shape change vectors r and their corresponding 95% credibility intervals are given. Separate multivariate MCMCglms were fitted for each species. Although the plastic response to temperature only correlates moderately between species, allometry, latitudinal differentiation and sexual shape dimorphism are rather conserved. Right: Magnitude of shape change in Procrustes units as represented by the summed length of all vectors (i.e. Procrustes distance) for each species and their respective angle in multidimensional space. *Sepsis punctum* responds more strongly to latitude, temperature and sex, whereas allometric responses are of similar magnitude in both species

4.1 | Plasticity and genetic differentiation in wing loading

Even though wing and thorax size clines differed qualitatively between species (Figure 2a and b), *S. punctum* as well as *S. fulgens* showed decreasing wing loading towards northern latitude (Figure 2c). As lower wing loading has been argued to provide better dispersal capacity in the cold (Azevedo et al., 1998; Frazier et al., 2008; Stalker, 1980) and the decline in wing loading with latitude was seen in both species, the pattern suggests some adaptive value to the observed responses (c.f. Endler, 1977). Moreover, given that a corresponding altitudinal gradient in wing loading has been described in sepsids (Rohner et al., 2015), and similar patterns are found within as well as between species of *Drosophila* (Azevedo et al., 1998; Rohner et al., 2018), these recurring patterns argue in favour of an adaptive scenario that is widespread among small dipterans. If so, wing clines will be generally steeper and hence may lead to quantitative and even qualitative differences compared to other

structural measures of size, implying that wings should be used with caution when studying body size variation along latitudinal clines (as is frequently done when assessing Bergmann's or James' rules; see e.g. Shelomi, 2012).

Based on earlier studies (e.g. Azevedo et al., 1998), we expected wing loading to increase with rearing temperature. When considering cold to moderate temperatures up to ca. 23°C, this was indeed supported. Given the genetic latitudinal clines in both species, this suggests cogradient variation across Europe. When considering the full reaction norm, however, we found very low wing loadings at the upper extreme of ca. 30°C in both species, resulting in a typical concave thermal reaction norm (Figure 2). One could thus speculate that selection on dispersal may not only drive decreasing wing loading in habitats that are too cold, but also in environments that are prone to over-heating. Indeed, in both cases heightened short-range dispersal capacity should increase the opportunity for thermoregulatory behaviour, suggesting adaptive benefits to the observed plasticity. Caution in this interpretation is needed, however, as hump-shaped

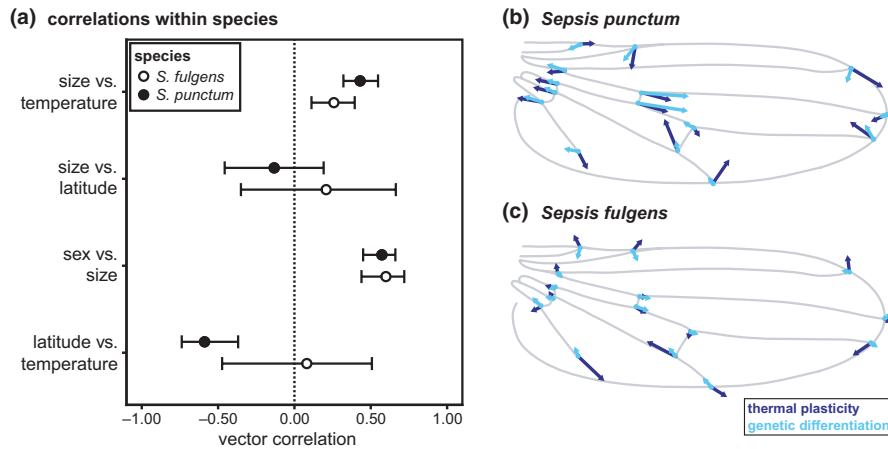


FIGURE 5 (a) Pairwise correlations (posterior modes \pm 95% credibility intervals) of shape change vectors observed within species in response to wing size (allometry), thermal plasticity, sexual dimorphism and latitude (genetic differentiation), based on 1,000 posterior estimates from Bayesian mixed-effects models. A correlation close to 1 would indicate strong congruence between induced phenotypic effects of two given variables, whereas a correlation close to zero would indicate unrelated effects. Shape deformations associated with temperature and latitude are illustrated in (b) and (c). Although thermal plasticity mirrors latitudinal genetic shape differentiation in *S. punctum* (b), the two do not correspond well in *S. fulgens* (c). (Note that because average temperature regimes are inversely correlated to latitude, the direction of shape change of latitude was reversed in (b) and (c).)

thermal performance curves are the rule rather than the exception, and therefore do not necessitate adaptive explanations (Angilletta, 2009; Chown et al., 2004). That is, organisms usually perform best at intermediate temperatures, and hence, phenotypes measured at the fringes of their intrinsic temperature tolerance are difficult to interpret, as they may be affected by biophysical constraints during development, resulting in trait decanalization. Moreover, the allometry between thorax length and body weight itself might be temperature-dependent. Our approach of using thorax length³ as an estimate of overall body mass might hence introduce artefacts. Consequently, for now, the putative adaptive value of thermal plasticity and its relationship to clinal variation in wing loading remains unclear but warrants further scrutiny.

4.2 | Plasticity and genetic differentiation in wing shape

Plastic as well as genetic responses in wing shape have been described in several *Drosophila* species. Although in these studies clinal variation in wing shape was often attributed to systematic differences in local selective regimes (e.g. Hoffmann & Shirriffs, 2002; Moraes & Sene, 2007; Pitchers et al., 2013), genetic differentiation along latitude is not ubiquitous and can arise due to drift and demography (Flatt, 2016; Schäfer et al., 2018). Here, we find latitudinal differentiation in *S. punctum* that corresponded with the plastic response. Northern *S. punctum* populations consequently show wing shapes similar to flies reared at cold temperatures. Such a cogradient is expected if the plastic and genetic responses are adaptive and driven by synergistic selection pressures. The observed pattern also fits at least phenomenologically to the predictions of genetic accommodation, whereby trait expression through ancestral plasticity becomes genetically canalized within populations (Crispo,

2008; Moczek, 2007; Waddington, 1952; West-Eberhard, 2003). Disentangling the potential effects of accommodation and other mechanisms that can generate cogradients is however difficult if information on the ancestral plasticity is lacking (Conover et al., 2009). Hence, we cannot argue with confidence that thermal plasticity contributed to the genetic differentiation in sepsid flies.

In contrast to *S. punctum*, we did not find latitudinal differentiation in *S. fulgens*. Note however that, albeit not significant, the shape changes associated with latitude correlated quite strongly between the species. The lack of significance in *S. fulgens* may thus be due to very small effect sizes and limited statistical power when regressing population means ($n = 9$) against latitude. Indeed, the latitudinal effect in *S. fulgens* also did not correlate with thermal plasticity. The absence of a cogradient in this species may in part be due to the weak statistical power, but could also suggest that the evolutionary processes driving wing shape differentiation are complex and not necessarily similar across closely related species. As the two focal species are rather similar in their distribution and ecology, it seems doubtful that selection on wing shape strongly contrasts between species. However, the potential for local adaptation greatly depends on the level of gene flow between populations (Crispo, 2008; Kirkpatrick & Barton, 1997). Previous data demonstrate strong neutral genetic differentiation between populations north and south of the Alps in *S. punctum*, as well as significant isolation by distance across the northern parts of the species' distribution (Puniamorthy, 2013). Because *S. punctum* is rather rare at high altitudes (Rohner et al., 2015), such genetic signatures are likely driven by limited gene flow across the alpine region, and may well relate to colonization histories after the last glaciation. In contrast, *S. fulgens* is common also at high altitudes (Rohner & Bächli, 2016; Rohner et al., 2015). Although we currently lack information on the underlying population structure of this species, we suspect that the Alps do not pose

a major barrier to gene flow, which would lead to only low levels of genetic differentiation across Europe (as in the closely related *S. cynipsea*: Kraushaar, Goudet, & Blanckenhorn, 2002). This could potentially hamper the potential for local adaptation and prevent the establishment of latitudinal clines (Kirkpatrick & Barton, 1997). The interspecific variation in wing shape clines could hence be explained by the underlying population structure and demography (cf. Flatt, 2016; Schäfer et al., 2018). If so, it would also imply that selection on wing shape is rather weak compared to selection on wing loading, for which we see latitudinal clines in both species.

4.3 | The effect of body size on phenotypic differentiation

Due to pronounced sexual size dimorphism and thermal plasticity of size, sexual shape dimorphism and temperature plasticity of wing shape are to some extent driven by allometry. Nevertheless, both sex and temperature explain shape variation independently of allometry, and these effects are qualitatively similar in the two species, despite that sexual size dimorphism is male-biased in *S. punctum* but female-biased in *S. fulgens*. This suggests that wing shape shows systematic differences between males and females independently of the direction of sexual size dimorphism, as has been found in other species (Gidaszewski, Baylac, & Klingenberg, 2009; Schäfer et al., 2018). In contrast, latitudinal clines were independent of body size (here estimated by thorax length), a pattern that was unexpected because both species show latitudinal patterns in wing size (Bergmann cline in *S. punctum*, and its converse in *S. fulgens*).

5 | CONCLUSIONS

Geometric morphometric studies offer great opportunities to investigate the relationship between plastic and genetic responses to common environmental drivers, potentially enabling predictions as to how complex phenotypes react to changing environments. Latitudinal population differentiation (south to north) in wing shape mirrored the plastic response to temperature (hot to cold) in *Sepsis punctum*, suggesting that phenotypic and genetic responses align and are driven by similar selection pressures and therefore adaptive. This phenomenologically fits with the patterns expected under genetic accommodation and suggests a moderating role for phenotypic plasticity in dictating the evolutionary response (Lande, 2009; Price et al., 2003). However, given our lack of an estimate of the ancestral form or magnitude of plasticity, the role of phenotypic plasticity in biasing or facilitating the observed genetic differentiation in this species awaits further scrutiny. However, although the effect of temperature on wing shape is somewhat conserved across the two closely related species with similar ecology, it was not related to clinal genetic differentiation in shape in *S. fulgens*. Thus, local adaptation and its relation to plasticity seems to some extent idiosyncratic and affected by various

factors, probably including the underlying population structure and variation in gene flow. However, in contrast to wing shape and body size, we find consistent clinal variation in wing loading, a pattern that is found repeatedly in various intra- as well as interspecific studies (Azevedo et al., 1998; Gilchrist & Huey, 2004; Rohner et al., 2015, 2018; Stalker & Carson, 1946; Starmer & Wolf, 1989). Wing loading might hence be under particularly strong and consistent selection (or under less restrictive genetic constraints) during adaptation to climate change in small pterygotes that depend on flight for thermoregulatory behaviour.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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