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RESEARCH ARTICLE

Mixed support for an alignment between phenotypic plasticity and genetic differentiation in damselfly wing shape

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Abstract

The relationship between genetic differentiation and phenotypic plasticity can provide information on whether plasticity generally facilitates or hinders adaptation to environmental change. Here, we studied wing shape variation in a damselfly (*Lestes sponsa*) across a latitudinal gradient in Europe that differed in time constraints mediated by photoperiod and temperature. We reared damselflies from northern and southern populations in the laboratory using a reciprocal transplant experiment that simulated time-constrained (i.e. northern) and unconstrained (southern) photoperiods and temperatures. After emergence, adult wing shape was analysed using geometric morphometrics. Wings from individuals in the northern and southern populations differed significantly in shape when animals were reared in their respective native environment. Comparing wing shape across environments, we found evidence for phenotypic plasticity in wing shape, and this response differed across populations (i.e. G×E interactions). This interaction was driven by a stronger plastic response by individuals from the northern population and differences in the direction of plastic wing shape changes among populations. The alignment between genetic and plastic responses depended on the specific combination of population and rearing environment. For example, there was an alignment between plasticity and genetic differentiation under time-constrained, but not under non-time-constrained conditions for forewings. We thus find mixed support for the hypothesis that environmental plasticity and genetic population differentiation are aligned. Furthermore, although our laboratory treatments mimicked the natural climatic conditions at northern and southern latitudes, the effects of population differences on wing shape were two to four times stronger than plastic effects. We discuss our results in terms of time constraints and the possibility that natural and sexual selection is acting differently on fore- and hindwings.

KEYWORDS

G×E, genetic differentiation, latitude, *Lestes*, phenotypic plasticity, photoperiod, wing shape

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1 | INTRODUCTION

Environmental variation across geographic gradients often drives patterns of local adaptation. Such evolutionary responses are widespread and are often repeatable (Blanckenhorn & Demont, 2004; Rudman et al., 2022). However, in addition to these evolutionary changes, organisms also have within-generation responses to environmental conditions. Such phenotypic plasticity is common and contributes significantly to intraspecific variation across environmental gradients. Moreover, there is often genetic variation for plasticity (genotype-by-environment interactions, $G \times E$), such that plasticity itself can evolve across populations. Phenotypic variation across geographic gradients is thus not only a function of genetic population differentiation, but also plasticity and population differences in the magnitude and direction of plastic responses. The interplay between plasticity, genetic population differentiation and $G \times E$ is vital for our understanding of (adaptive) evolution. One reason for such an alignment could be that adaptation is constrained to occur along dimensions for which there is additive genetic variation, and because of biases in development, genetic variation and plasticity tend to occur along these shared dimensions, see for example Draghi and Whitlock (2012). It might also be that developmental plasticity could hinder or promote adaptation along certain dimensions by providing benefits to individual genotypes that exhibit a certain kind of plasticity that puts them closer or further away from the new phenotypic optimum: assuming that the environment has changed, see for example, Price et al. (2003). In this case, if genetic differentiation and plasticity are adaptive, they are expected to point into the same direction. If so, such an alignment could speed up adaptation to new environmental conditions (Lande, 2009; Levis & Pfennig, 2016; Price et al., 2003, but see Price et al., 2003; Whitlock, 1996), and contemporary plasticity could be used to predict future evolutionary responses to environmental changes (e.g. Rohner & Moczek, 2020). However, an alignment between genetic divergence and plasticity in wing shape could also be non-adaptive and reflect constraints. This could occur if the developmental system channels variation down certain developmental pathways/phenotypic dimensions (Draghi & Whitlock, 2012; Uller et al., 2018), or when ancestral plasticity is maladaptive, that is opposes the direction of selection. However, how often plasticity and population differentiation evolve to align remains unclear. Here, we study damselfly wing shape variation to explore how latitudinal population differentiation relates to phenotypic plasticity.

In pterygotes (i.e. flying insects), wings are important for adult individual performance in almost all aspects of the insect lifestyle, including dispersal, migration, predator avoidance and sexual selection (Flockhart et al., 2017; Johansson et al., 2009; Outomuro et al., 2016; Outomuro & Johansson, 2015). This has led to the evolution of large interspecific population differences in wing shape among species with different ecologies, but also variation in morphology within and among populations (Gilchrist & Huey, 2004; Rohner et al., 2019). For instance, many studies have found latitudinal variation in wing size and shape (Azevedo et al., 1998; Gilchrist & Huey, 2004; Rohner

et al., 2019). At the same time, wing shape is also affected by the temperature experienced during development (i.e. thermal plasticity, Debat et al., 2003). Since ambient temperature affects flight through changes in wingbeat frequency and other biomechanical effects, selection is likely to act on wings and associated structures (Unwin & Corbet, 1984). Latitudinal differentiation in wing morphology, as well as thermal plasticity, is thus often argued to represent adaptive responses to optimize flight at different temperatures (Frazier et al., 2008; Stalker, 1980). For example, *Drosophila melanogaster* develops narrower wings at colder temperatures (Imasheva et al., 1995, but see Przybylska et al., 2014 for the opposite pattern) which may increase performance at lower temperature (Frazier et al., 2008). We note, however, that flies have one pair of wings, whereas many other insects have two, and hence, the optimal wing shape might differ among taxa. If wing shape is an adaptation to physical environmental factors in adults such as terrestrial air temperature, then photoperiod and temperature might be important environmental cues in the larval stage working as proximate factors determining wing shape in the adult stage. In addition to selection from the physical environment such as temperature, local selection pressures on wing shape resulting from biotic factors such as predation and sexual selection (e.g. Outomuro & Johansson, 2011) might also differ systematically along latitude within species, but may not be paralleled across species with different ecology. Hence, it cannot be excluded that abiotic environmental cues such as temperature and photoperiod can be used to predict future biotic conditions. Finally, wing shape might also be affected by temperature-dependent biochemical and developmental rate processes (de Jong & van der Have, 2009), and therefore, some of the wing shape variation observed at different temperatures might not be an adaptation.

Given the various selection pressures on wings and the common genetic and plastic responses, wing shape is an ideal trait to study how plasticity and genetic differentiation interact. A recent study by Rohner et al. (2019) on wing shape in two species of sepid flies along a latitudinal gradient showed that genetic population differentiation and phenotypic plasticity were aligned in one of the species but not in the other. Similarly, Azevedo et al. (1998) showed that genetic differentiation and phenotypic plasticity of wing aspect ratio in *Drosophila melanogaster* showed the same response with decreasing temperature along a latitudinal gradient. Rapid adaptation in dung beetle wing shape was also partially related to thermal plasticity (Rohner & Moczek, 2020). By contrast, thermal plasticity was not related to latitudinal variation in wing shape in the yellow dung fly on three continents (Schäfer et al., 2018). Similarly, Gilchrist and Huey (2004) found no strong evidence of an alignment between genetic differentiation and phenotypic plasticity of wing loading (i.e. relationship between wing area and body weight) in South American populations of *Drosophila subobscura*. Given the mixed results of previous studies and the polygenic basis of wing shape variation (Carreira et al., 2011), it is unclear whether genetic and plastic variation generally align in insect wing shape, especially because most studies have been performed on dipterans with similar flight ecologies.

Temperature is an important environmental cue in seasonal environments. However, adaptive plastic responses to seasonal environments are also often cued by photoperiod (i.e. day length)—a much more reliable indicator of seasonality and season length than temperature (Bradshaw & Holzapfel, 2017). Photoperiod is especially important for insects with long and complex life cycles because declining season lengths towards the North restrict larval growth and consequently adult size and morphology (de Jong & van der Have, 2009; Rowe & Ludwig, 1991; Sniegula et al., 2016). In organisms with an obligate one-year life cycle, this leads to a decrease in adult size with increasing latitude (Rowe & Ludwig, 1991). In these species, adaptive adjustments to wing shape may thus also be tied to photoperiod cues and not just rearing temperature (as in many other species, e.g. Zeender et al., 2019). The shorter time available for growth and development could also affect wing shape independently of a temperature effect, since size at maturity can scale allometrically with wing shape (Outomuro et al., 2013, 2021). Hence, for an appropriate estimate of wing shape variation along a latitudinal gradient, organisms must be reared at conditions simulating both temperature and photoperiod conditions at the latitude of origin.

The damselfly *Lestes sponsa* (Hansemann, 1823) is an excellent non-model organism to study whether genetic population differentiation and phenotypic plasticity in wing shape are aligned along a latitudinal gradient. This species has an obligate one-year life cycle, and northern populations develop faster and emerge at a smaller size (Sniegula et al., 2016) and are thus time-constrained. In line with latitudinal differences in life history, wing shape differs between field-collected individuals along a latitudinal gradient, although latitudinal effects account for <4% of the total wing shape variation (Outomuro et al., 2021). In addition, studies have shown that natural selection, estimated from data on adult mortality, favours long and slender forewings and short and broad hindwings (Outomuro et al., 2016). By contrast, sexual selection, estimated from data on mating success, favours short and broad forewings and narrow-based hindwings (Outomuro et al., 2016). Hence, variation in wing shape is evident in this *Lestes sponsa* system, and some of this variation relates to fitness. However, the relationship between genetic population differentiation and phenotypic plasticity in wing shape remains unexplored.

Using reciprocal transplant experiments, a recent study on life history traits in *L. sponsa* found that the direction of multivariate plasticity was aligned with latitudinal population divergence and standing genetic (co)variation (the G-matrix) (Johansson et al., 2021). This alignment was only found under time-constrained conditions, that is under temperature and photoperiod conditions simulating northern latitudes (Johansson et al., 2021). This suggests that the relationship between genetic variation and plasticity may itself be environment dependent, and here, we thus set out to characterize the corresponding relationships for wing shape.

Our first objective was to test for latitudinal wing shape variation in *L. sponsa*. We did this by raising *L. sponsa* in the laboratory simulating the native natural temperature and photoperiod conditions along the latitudinal gradient. Since time constraints are strong and

temperature is colder in the North, we predicted narrower wings at northern latitudes as has been found in *D. melanogaster* (Imasheva et al., 1995), but we note that *Drosophila* have one pair of wings, whereas *L. sponsa* has two pairs. We also suggest two alternative predictions. In the first alternative one, we predict short and broad forewings and narrow-based hindwings in males in the North. The reason is that components of sexual selection, such as mating intensity, are higher in the North in this species (Golab et al., 2019), and that sexual selection has been shown to favour such wing shape in males (Outomuro et al., 2016). However, we note that sexual selection includes more components than mating intensity. In the second alternative one, we predict that northern populations should have more pointed and slender wings since such wings are favoured by dispersal (Wootton, 1991, 1992): *L. sponsa* disperses towards the North since the last glacial period in Europe.

Our second objective was to examine the relationship between genetic population differentiation and phenotypic plasticity in wing shape. We did this by raising a northern and a southern population under photoperiods and temperatures simulating time-constrained (northern) and non-time-constrained (southern) conditions. Our past studies on *L. sponsa* have found genetic differentiation in adaptation and adaptive plasticity in life history traits of populations along the latitudinal gradient in Europe (Sniegula et al., 2016; Śniegula et al., 2014). Thus, if population genetic differentiation and phenotypic plasticity of wing shape reflect adaptive responses the physical or biotic environmental difference along the latitudinal gradient, we expect genetic differentiation and phenotypic plasticity to be aligned. Because time constraints are stronger and temperature less variable during the growth season in the North, we expected northern populations to show reduced plasticity and a stronger alignment between plasticity and genetic population differentiation relative to the southern population.

2 | METHODS

2.1 | Field collection and laboratory experiment

The study species *L. sponsa* is widespread throughout Europe except for the southern part of the Mediterranean peninsulas (Boudot & Kalkman, 2015). It has a 1-year obligate life cycle and overwinters at the egg stage (Corbet, 1956). The eggs hatch in spring, and the aquatic larval stage lasts for 2–3 months after which the terrestrial adult stage has a lifespan of about 1 month (Corbet, 1956). All growth occurs during the larval stage, but adults can increase their mass by feeding (Hyeun-Ji & Johansson, 2016). Hence, photoperiod and water temperature during the larval stage have a great impact on adult life history and morphology.

To estimate plastic and genetic responses, several *L. sponsa* populations were reared under laboratory conditions. A detailed description of the experimental procedures is provided by Johansson et al. (2021). In brief, eggs were sampled from mated wild-caught females (forming full-sib families) caught at three latitudes in Europe:

Northern Sweden (northern: 66° N; $n = 34$ females), Central Sweden (central: 59° N; $n = 36$), and Northwestern Poland (southern: 54° N; $n = 38$) in late summer 2018. We sampled three locations for northern Sweden, two for central Sweden and two for north-western Poland, see [Table S1](#). Although Poland is not in Southern Europe, we will hereafter refer to these populations as north, central and south (i.e. in a latitudinal context) for simplicity. We currently lack data on the level of genetic divergence among these populations, but life history traits of common garden-reared individuals differ significantly between populations of *L. sponsa* along the latitudinal range used in our study, suggesting genetic population differentiation (Johansson et al., 2021; Sniegula et al., 2016).

Upon capture, mated females were brought to the laboratory and placed in plastic cups lined with moist filter paper, wherein they laid eggs. The eggs were thereafter subjected to native environmental conditions simulating the autumn and winter temperatures and photoperiod at the latitude of collection. After the termination of the winter conditions, eggs were subject to temperatures and photoperiods simulating the spring conditions at the latitude of collection. The eggs hatched about 10 days after the spring conditions started, and the hatched larvae were reared using brine shrimp *ad-libitum* until emergence. During their last instar, larvae were also fed three standard-sized live midge larvae every Monday, Wednesday and Friday.

During the whole period of raising the larvae, photoperiod and temperature were changed on a weekly basis simulating the progression of the season. For the first objective, comparing phenotypic wing shape between northern, central and southern populations, larvae were reared in their native photoperiod and temperature. For the second objective, comparing genetic differentiation and phenotypic plasticity in wing shape, larvae from northern and southern populations (but not from the central population) were reared in a common garden transplant experiment design using full-sibs, that is a fully factorial (2×2) experimental design. Hence, full-sibs of northern and southern populations experienced short and long seasons (as simulated by photoperiod and temperature). We assumed that offspring within clutches are full-sibs, but note that some clutches (about 40%) in *L. sponsa* might not be full-sibs (Johansson et al., 2020). Emerged adults were preserved in alcohol for later measurements of wing shape, and only males were used.

2.2 | Geometric morphometric analysis

To analyse wing shape, we applied a geometric morphometric framework. The right fore- and hindwings were removed and placed underneath a glass slide. Photographs of the flattened wings were taken with a Panasonic DMC-FZ300 camera mounted to a stand, see the Electronic Supplement for examples. On the photographs, we digitized 13 landmarks ([Figure 1](#)) using tpsDig version 2.31 (Rohlf, 2018). Landmark nr. 10 was a semi-landmark ([Figure 1](#)). Fore- and hindwing landmark coordinates were subject to a full Procrustes

superimposition using the function `gpagen` of the R-package `geomorph` version 4.0.0 (Adams et al., 2021). The position of the semi-landmark was optimized by minimizing bending energy between the reference shape and each individual shape. Separate analyses were run for fore- and hind wings since our purpose was not to compare differences between fore- and hind wings. From the landmark coordinates for each individual wing, wing centroid size was calculated as the square root of the sum of squared distances between each individual landmark and the centroid of the shape configuration. Log wing centroid size was thereafter used as an estimate of individual size (see for instance Klingenberg, 2016).

2.3 | Latitudinal variation in wing shape

To test for phenotypic variation between the three sampled latitudinal locations, we first compared wing shape between southern, central and northern populations when reared in their respective native environment. For this analysis, we used Procrustes ANOVAs with residual randomization permutation (using type II sums of squares) as implemented in the R-package `geomorph` (Adams et al., 2021) using the function `procD.lm`. We used log centroid size, population (northern, central or southern latitude), full-sib family identity nested within population and all possible interactions as independent variables. Non-significant interaction terms were removed from final models. Past studies using the same locations as in this study showed no population-related genetic differences or difference in genetic variance in estimated life history and morphology traits within latitudes (Sniegula et al., 2016; Sniegula et al., 2018). We therefore removed the locality effect from all models to increase the power of comparisons. Procrustes ANOVAs were performed separately for fore- and hindwings. Because the three populations were reared in their local environment, latitudinal wing shape variation approximates the phenotypic (as opposed to the genetic) latitudinal variation expected in the field.

2.4 | Alignment between population differentiation and phenotypic plasticity

Next, we tested whether genetic differentiation and phenotypic plasticity of wing shape were aligned. For this analysis we excluded the central population and focused on the southern and northern populations that were both reared under constrained (northern) as well as unconstrained (southern) seasonal conditions. As for the phenotypic comparison described above, we removed locality in the model since past studies have shown no genetic differentiation between localities at each latitude (Sniegula et al., 2016; Sniegula et al., 2018). Thus, to test for genetic and plastic variation, we used Procrustes ANOVAs including the effects of log centroid size, population (North vs. South), rearing environment (short versus long season, i.e. constrained and unconstrained photoperiod and temperature conditions), full-sib family identity nested within population,

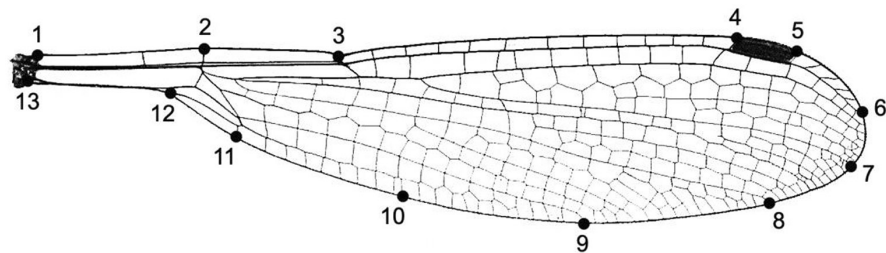


FIGURE 1 Wing showing the 13 landmarks used in morphometric analysis. Landmark 10 was a sliding landmark (semi-landmark).

and all interactions as independent variables. Non-significant interaction terms were removed.

Because Procrustes ANOVAs indicated significant population-by-environment interactions for fore- and hindwings (see Results), we fitted additional multivariate models separately for each population and environment combination, to test for differences in the direction and magnitude of shape changes. To do so, we removed allometric (i.e. size-related) variation from our entire data set by taking the residuals of a multivariate regression of shape on log centroid size (note that we did not find evidence for differences in allometry among populations or environments in fore- or hindwings using Procrustes ANOVAs). To derive the shape deformation vectors associated with the effect of the rearing environment, we fitted wing shape as a function of full-sib family and rearing condition for each population separately as:

$$Y_{i,j,k} = \beta_0 + E_i + F_j + \epsilon_k$$

where Y indicates a matrix of 26 allometry-adjusted wing shape variables, β_0 is a vector of intercepts, E denotes the effect of the rearing environmental treatment, F is a vector indicating family differences, and ϵ is the error term. The vector of partial coefficients E then describes the direction and magnitude of shape changes driven by changes in photoperiod and temperature.

To quantify population differentiation under short and long seasons (North vs. South), we fitted wing shape as a function of population and full-sib family for each environment separately as:

$$Y_{i,j,k} = \beta_0 + P_i + F_{j(i)} + \epsilon_k$$

where P indicates the population and F indicates the family nested within population. The vector of partial regression coefficients P represents the direction and magnitude of population differences in the respective rearing environment. Next, we extracted the vectors associated with the fixed effects of rearing environment treatment (E) in both populations and the effect of population (P) under short and long seasons to compare plastic responses to genetic population differentiation. This allowed us to assess all pairwise alignments between all four vectors. Specifically, we compared (i) plastic shape changes in the northern population, (ii) plastic shape changes in the southern population, (iii) the genetic responses found under simulated short (northern) season length and (iv) the genetic responses found under simulated long (southern) season length. The alignment of shape deformation vectors was quantified using pairwise vector correlations as:

$$r_{V_i, V_j} = \frac{V_i \cdot V_j}{|V_i| \times |V_j|}$$

where the numerator denotes the dot product of the two vectors of coefficients V_i and V_j (i.e. P or E in our case), and the denominator represents the product of their norms (Zelditch et al., 2012). We also calculated the magnitude of each vector ($|V|$) to quantify the total amount of shape change associated with each fixed effect. We calculated bias-corrected and accelerated bootstrap (BCa) confidence intervals for vector correlations and vector norms based on 9999 non-parametric bootstrap replicates.

3 | RESULTS

3.1 | Latitudinal differences in fore- and hindwing shape

There were significant differences in forewing shape between the southern, central and northern populations when they were reared in their native environment (Procrustes ANOVA, Table 1). Wings from the North had a more compressed distal part, and the anterior part of the petiole extending towards the body (Figure 2). Wings from the South had a blunter wing tip and a broader and shorter petiole, resulting in a somewhat more elongated shape compared to those in the North. The central wings were not intermediate in shape as they had a very narrow shape overall, with a broader tip (Figure 2). For hindwings, the shape differences between the northern, central and southern populations were also significant and qualitatively similar, but less pronounced compared to the forewings (Figure 2). Thus, hindwings showed less variation than forewings (Figure 2).

3.2 | G × E interactions in forewing shape

Our reciprocal transplant experiment allowed us to assess plastic responses across the northern and the southern population. Forewing shape showed a significant centroid size effect, differed between populations and rearing environments and showed pronounced genetic variation within populations (full-sib family nested within population) (Figure 3, Table 2a). Moreover, there were also significant differences in the way each population responded to season length manipulations (population-by-environment interaction, Figure 3,

TABLE 1 Procrustes ANOVAs comparing fore- and hindwing shape between south, central and north populations.

	Df	SS	MS	R ²	F	Z	p
(A) Forewing Procrustes ANOVA (type II SS)							
Log centroid size	1	0.0006	0.0006	0.01	1.68	1.13	0.134
Population	2	0.0039	0.0019	0.04	3.61	3.00	0.003
Family nested within population	85	0.0463	0.0005	0.50	1.59	4.38	0.001
Residuals	112	0.0384	0.0003	0.41			
Total	200	0.0927					
(B) Hindwing Procrustes ANOVA (type II SS)							
Log centroid size	1	0.0008	0.0008	0.01	2.03	1.50	0.073
Population	2	0.0043	0.0021	0.04	4.39	3.81	0.001
Family nested within population	84	0.0411	0.0005	0.42	1.18	1.73	0.040
Residuals	113	0.4696	0.0004	0.48			
Total	200	0.0982					

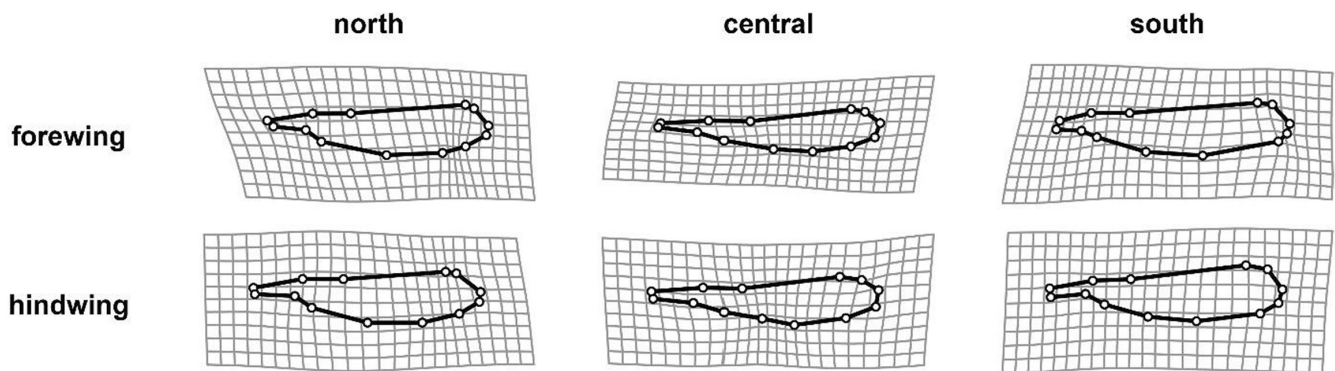


FIGURE 2 Shape differences between populations reared in their local environment. Shape deformations are magnified 15-fold and indicate relative differences to the overall mean shape.

Table 2a). To closer inspect this $G \times E$ interaction, we fitted separate models for each rearing environment and population combination.

We found that this interaction was driven in part by a much stronger plastic response in the northern population (vector norm $|E| = 0.85 \times 10^{-2}$ [0.54×10^{-2} , 1.12×10^{-2}] 95% BCa confidence limits) compared to the plastic response in the southern population ($|E| = 0.47 \times 10^{-2}$ [0.34×10^{-2} , 0.54×10^{-2}]). In fact, when analysing this in a separate Procrustes ANOVA, plasticity to season length became non-significant in the southern population ($F_{1,114} = 1.92$, $p = 0.077$; Table S2). In addition, we also recovered a poor alignment when comparing the direction of the plastic response of the northern population to the one in the southern population ($r = 0.20$ [-0.31 , 0.64]). Plasticity in the northern population mostly related to the relative width of the wing and the position of landmark 3 along the wing edge (Figures 3a and 4a). By contrast, in the southern population, plastic changes mostly focused on the wing tip (Figures 3a and 4a). This suggests that seasonal plasticity has evolved both in direction and magnitude. By contrast, population differences were much more similar across rearing conditions, in both magnitude (short season: $|P| = 3.66 \times 10^{-2}$ [2.52×10^{-2} , 4.91×10^{-2}], long season: $|P| = 3.12 \times 10^{-2}$ [1.61×10^{-2} , 3.78×10^{-2}]) and direction ($r = 0.61$ [-0.03 , 0.89], see Figures 3a and 4, Table 3).

The significant $G \times E$ interaction complicates the comparison between plastic and genetic responses. We thus present all pairwise vector correlations and vector norms (i.e. the strengths of shape changes) in Table 3a and Figures 3 and 4 (also see Figure S1). Comparing the correlations between the two plasticity and the two genetic vectors rendered two main results: firstly, the northern population's plastic response correlated with the population differences that were found under short-season environments ($r = 0.44$ [0.00 , 0.83]) but not in a long-season environment ($r = -0.11$ [-0.68 , 0.48]). Secondly, plasticity in the southern population was clearly distinct from all other vectors and tended to be in the opposite direction to the population differences found in both environmental treatments ($r = -0.51$ [-0.85 , 0.058] and $r = -0.67$ [-0.89 , -0.50] respectively). Taken together, this indicates that plasticity has evolved across populations and that plasticity is only partially aligned with genetic latitudinal differences.

3.3 | $G \times E$ interactions in hindwing shape

Similar to the forewings, Procrustes ANOVA revealed significant effects of log centroid size, population, rearing environment, family

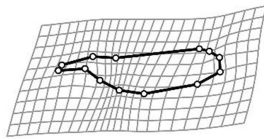
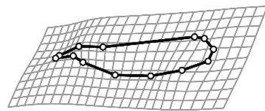
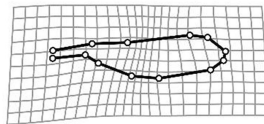
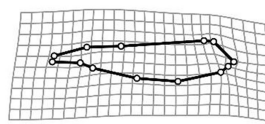
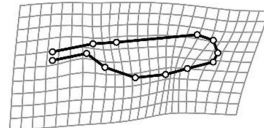
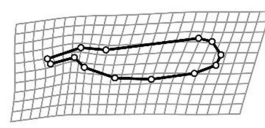
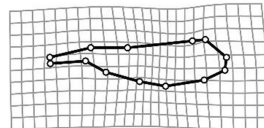
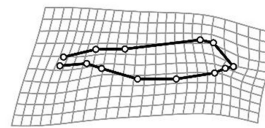
(a) forewingpopulation differentiation
short season (x4)population differentiation
long season (x4)plasticity
northern population (x10)plasticity
southern population (x10)**(b) hindwing**population differentiation
short season (x4)population differentiation
long season (x4)plasticity
northern population (x10)plasticity
southern population (x10)

FIGURE 3 Partial effects of population (North vs. South) and rearing environment (short vs. long season) on fore- and hindwing shape shown as deformation grids. The grids show deformation caused by a shift from the northern population to the southern one, or the northern environment to the southern one.

nested within population and the population-by-environment interaction term (Figure 3b, Table 2b: note that the environment effect was $p = 0.054$). This interaction was again driven in part by a much stronger plastic response in the northern population ($|E| = 0.85 \times 10^{-2}$ [0.57×10^{-2} , 1.02×10^{-2}]) compared with the plastic response in the southern population ($|E| = 0.52 \times 10^{-2}$ [0.37×10^{-2} , 0.6×10^{-2}]). In addition, we found a poor alignment between plasticity in the northern and the southern populations ($r = -0.17$ [-0.60 , 0.19]). Plasticity in the northern population mostly related to the relative width of the wing, whereas in the southern populations, rearing environment affected the width of the wing base and the wing tip (Figures 3b and 4, Figure S1). This suggests that, similar to the forewing, seasonal plasticity has evolved both in direction and in magnitude. Population differences, contrary to forewings, were not aligned across environments ($r = 0.12$ [-0.5 , 0.52] see Figures 3b and 4) but were similar in magnitude (short season: $|P| = 2.37 \times 10^{-2}$ [1.49×10^{-2} , 3.66×10^{-2}], long season: $|P| = 2.45 \times 10^{-2}$ [1.71×10^{-2} , 3.99×10^{-2}]).

In contrast to the forewing, we found overall much weaker correlations across all comparisons for the hindwing (Table 3,

Figure S2). The northern population's plastic response did not correlate with the genetic effect in a short-season environment ($r = 0.24$ [-0.29 , 0.69]) and tended to oppose the genetic effect in a long-season environment ($r = -0.36$ [-0.82 , -0.03]). Plasticity in the southern population tended to point in the opposite direction of the population differences found in both environmental treatments (short season: -0.28 [-0.67 , 0.09], long season: -0.34 [-0.77 , 0.08]).

4 | DISCUSSION

We found phenotypic differences in male wing shape among populations, although population differentiation did not follow a simple linear relationship with latitude. The reciprocal common garden transplant experiments confirmed that wing shape differences across populations were composed of genetic and plastic responses to the juvenile rearing environment. Interestingly, we found evidence that phenotypic plasticity is evolving both in magnitude and direction across populations: the plastic response was much stronger in the northern compared to the southern population and differed in its direction. Some comparisons between genetic and plastic responses indicated an alignment; however, many comparisons indicated that plasticity was not related to genetic differences, or that direction of plasticity pointed in opposite directions (Table 3). Given the context dependency of these alignments, support for an alignment between plasticity and genetic differentiation is mixed at best. We discuss the potential adaptive and non-adaptive causes and consequences of these findings.

4.1 | Wing shape showed non-linear latitudinal differences affected by genetic and plastic components

Phenotypic responses of animals to latitudinal gradients are often expected to be linear, especially in obligately univoltine insects (Roff, 1980). In contrast to this expectation, wings in the central population were not intermediate in shape between the northern and southern populations, suggesting that there is no linear latitudinal cline in wing shape within the studied range. A previous study on field collected adults of *L. sponsa* from 17 populations across a latitudinal gradient spanning from southern to northern Europe showed a U-shaped instead of a linear latitudinal pattern for wing size and body size (Outomuro et al., 2021). The study also showed linear and non-linear latitudinal variation in wing shape, but the contribution of these clinal patterns to total wing shape variation was relatively small. The authors speculated that latitudinal variation in natural and sexual selection might be responsible for the lack of a more pronounced linear cline, whereas aerodynamic constraints might account for the limited amount of wing shape variation overall (Outomuro et al., 2021). That study did however not account for environmental variation in wing shape

TABLE 2 Procrustes ANOVAs for each origin and rearing condition combination for fore- (A) and hindwings (B) respectively.

	Df	SS	MS	R ²	F	Z	p
(A) Forewing Procrustes ANOVA (type II SS)							
Log centroid size	1	0.0015	0.0015	0.01	3.95	2.84	0.002
Population	1	0.0027	0.0027	0.02	4.65	2.60	0.003
Environment	1	0.0011	0.0011	0.01	2.85	2.07	0.022
Population × environment	1	0.0012	0.0012	0.01	3.35	2.38	0.007
Family nested within population	68	0.0395	0.0006	0.31	1.57	5.40	<0.001
Residuals	209	0.0772	0.0004	0.60			
Total	281	0.1280					
(B) Hindwing Procrustes ANOVA (type II SS)							
Log centroid size	1	0.0027	0.0027	0.02	6.47	3.26	<0.001
Population	1	0.0021	0.0021	0.02	3.31	2.33	0.013
Environment	1	0.0009	0.0009	0.01	2.07	1.67	0.054
Population × environment	1	0.0019	0.0019	0.01	4.65	3.12	<0.001
Family nested within population	68	0.0435	0.0006	0.30	1.55	4.87	<0.001
Residuals	212	0.0875	0.0004	0.61			

which we were able to do in the present study by using a common garden transplant approach. This allowed us to explore the genetic differences and plasticity in wing shape between the northern and southern populations (excluding the central population, which was reared in a different environmental treatment). When rearing animals in a common garden environment, we found evidence for genetic differentiation in wing shape between southern and northern populations, with wings in the North being on average somewhat shorter and pointier at the tip. Thus, the phenotypic wing differences along the latitudinal gradient differed from those found by Imasheva et al. (1995) on *D. melanogaster*, since we did not find a narrower wing shape in the North. Because wings with a pointier tip are usually better for long-distance flight in odonates (Wootton, 1991, 1992), these phenotypic changes may alter dispersal capacity, although the adaptive, if any, impact is unclear. For instance, due to the tendency of odonates to expand their ranges northwards (Hickling et al., 2005; Termaat et al., 1984), the northern population may receive more immigrants. Because migrating individuals are expected to be better dispersers (e.g. by having pointier wings), increased dispersal capacity of northern populations may be driven by an influx of dispersers from more southern populations.

Interestingly, population differences between the northern and southern populations were similar across the two rearing environments for forewings, but not for hindwings. Since we lack aerodynamics studies of the flight of *L. sponsa*, we can only speculate on whether these differences have functional implications. Previous studies have shown that different selection pressures act on fore- and hindwings in *L. sponsa* (Outomuro et al., 2016) and other odonates (Johansson et al., 2009; Kuchta & Svensson, 2014; Outomuro et al., 2014; Outomuro & Johansson, 2019), and the stronger alignment for forewings suggests similar and stronger selection on the front wings compared with hind wings.

4.2 | Phenotypic plasticity was stronger in the North

Our results showed a stronger plastic response in wing shape in the northern compared to the southern populations. This was surprising as we would have expected less plasticity in northern populations, since the time constraints are higher in the North, that is imposing strong stabilizing selection also affecting plasticity. Even though thermal breadth experienced by an organism shows a positive relationship with latitude (Sunday et al., 2019), within-generation temperature variation experienced during the growth season decreases towards the North in univoltine species (Nilsson-Örtman et al., 2012). Thus, temperature variation is lower in the North and hence should not select for plasticity. Many studies have found phenotypic plasticity in wing size, wing loading and wing aspect ratio in populations along a gradient (e.g. Azevedo et al., 1998; Gilchrist & Huey, 2004), and the few that have focused on wing shape plasticity along a latitudinal gradient have done so usually focusing on plasticity at different temperatures (Rohner et al., 2019). It is therefore difficult to compare our result on plasticity along the latitudinal gradient quantitatively since our plasticity treatment included temperature and photoperiod: two very important cues that determine growth and development of insects evolving under time constraints (Tauber et al., 1986). Of these two, photoperiod is probably the most important cue affecting development since photoperiod shows very predictive changes within a year compared to temperature (Bradshaw & Holzapfel, 2017). However, other abiotic and biotic factors may also influence plastic and genetic responses in wing shape along the latitudinal gradient (e.g. predation risk, interspecific competition and air temperature).

One reason for the higher plasticity in the northern population could be that northern populations are receiving many immigrants (see above). This would bring new genetic variation in the

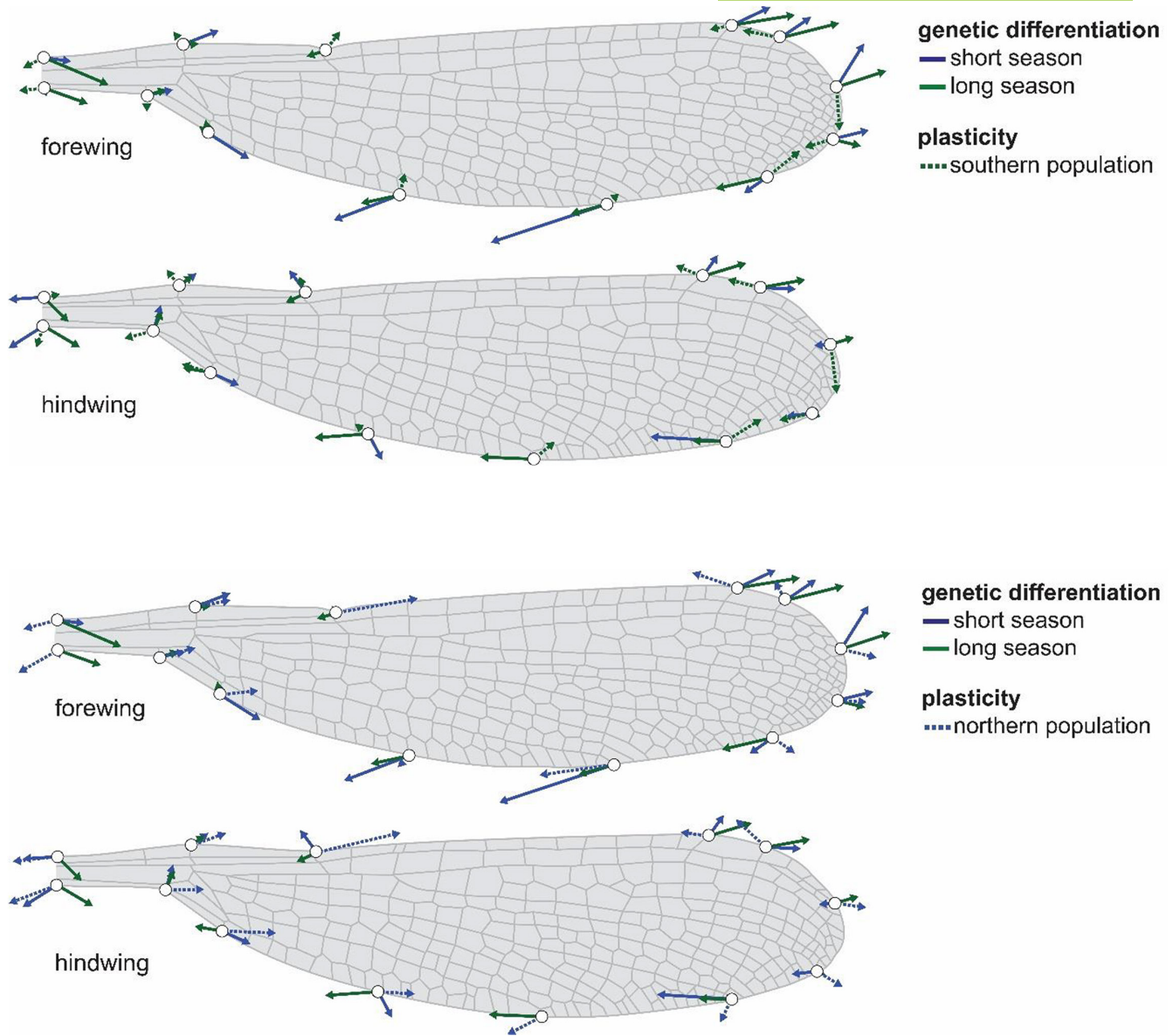


FIGURE 4 Alignment between plastic and genetic responses in fore- and hindwing shape (North vs. South). Population differences are magnified four times; plasticity is exaggerated 15 times.

population which could result in higher plasticity. Such patterns have been predicted by theoretical models and, for example Chevin and Lande (2011) showed that higher plasticity might evolve at the edges of the geographic range. There is also empirical support for such pattern, for example Lind et al. (2010) found that the phenotypic plasticity was positively correlated with gene flow from other populations in an island system.

4.3 | Mixed evidence for an alignment between genetic differentiation and phenotypic plasticity

If plasticity and genetic responses represent adaptations to the same environmental variable, genetic population differences and plasticity are expected to be aligned. For forewings, we found a (partial)

alignment between the northern population's plasticity and genetic population differentiation under constrained rearing conditions, such that wings showed a pointier wing tip and a shorter and slightly broader overall shape. By contrast, we did not find an association between the southern population's plasticity and genetic population differences in either environment. The alignment between plasticity and genetic population differences was much stronger in the northern population compared with the southern population. This could suggest that plasticity in the northern populations has evolved and thereby has caused the alignment with the genetic response, or that the plasticity in southern populations has evolved due to other evolutionary pressures that are not cued by time constraints.

It is difficult to assess the potential adaptive implications of shorter yet pointier wings in high-latitude environments without proper aerodynamic analyses. Based on previous studies in this

TABLE 3 Pairwise vector correlations between the effects of environment (plasticity) and population on fore- (A) and hindwing (B) shape.

	Plasticity		Population differences	
	Northern population	Southern population	Short season	Long season
(A) Forewing				
Plasticity				
Northern population	1.00	0.20 [-0.31, 0.64]	0.44 [0.00, 0.83]	-0.11 [-0.68, 0.48]
Southern population		1.00	-0.51 [-0.85, 0.05]	-0.67 [-0.89, -0.50]
Population differences				
Short season			1.00	0.61 [-0.03, 0.89]
Long season				1.00
Vector norm ($\times 10^2$)	0.85 [0.54, 1.12]	0.47 [0.34, 0.54]	3.66 [2.52, 4.91]	3.12 [1.61, 3.78]
(B) Hindwing				
Plasticity				
Northern population	1.00	-0.17 [-0.60, 0.19]	0.24 [-0.29, 0.69]	-0.36 [-0.82, -0.03]
Southern population		1.00	-0.28 [-0.67, 0.09]	-0.34 [-0.77, 0.08]
Population differences				
Short season			1.00	0.12 [-0.50, 0.52]
Long season				1.00
Vector norm ($\times 10^2$)	0.85 [0.57, 1.02]	0.52 [0.37, 0.6]	2.37 [1.49, 3.66]	2.45 [1.71, 3.99]

Note: Vector norms (i.e. lengths, multiplied by 10^2) and corresponding bias-corrected and accelerated bootstrap (BCa) confidence intervals are provided for each vector separately.

study system, a shorter and broader forewing shape is favoured by sexual selection in males (Outomuro et al., 2016). It has been suggested that sexual selection is more intense at higher latitudes due to shorter mating seasons, resulting in increased male–male competition (Golab et al., 2019) and large investment in armaments that increased mating success (Sniegula et al., 2017). Thus, our results could suggest that the observed genetic and plastic changes in response to time constraints may be a result of stronger sexual selection in the North, ultimately leading to an alignment. If this interpretation is correct, it also suggests that photoperiod might act as a cue informing organisms about selection pressures caused by biotic factors. Besides forewings being shorter and broader, the alignment for forewings also showed a pointier wing tip. Morphological variation on wing tips is generally important for flight aerodynamics (Shyy et al., 2016; Yanjuan, 2022), and similar changes have been correlated with migratory ability in birds, bats and dragonflies (Bowlin & Wikelski, 2008; Johansson et al., 2009; Voelker, 2001).

The alignment between plasticity in the northern population and genetic differentiation under short-season-rearing conditions further corresponds to a recent finding by Johansson et al. (2021). That study used the same data set and found that plasticity and genetic differentiation in life history traits were aligned, but only under time-constrained rearing conditions. The similar observations for life history traits and wing shape may indicate that these are indeed general patterns driven by strong and consistent selection pressures, or that the alignment found in wing shape may be driven by pure growth and developmental constraints (e.g. due to pleiotropy). An analysis combining wing shape and life history traits would

be very interesting, but such a multivariate approach will require a much larger data set compared to what we have available and is thus beyond the scope of this article.

4.4 | Population differentiation has stronger impact on wing shape than plasticity

The overall wing shape change (i.e. the length of deformation vectors) due to plasticity was two to four times smaller than the effect of population differentiation. This result contrasts with the findings of a recent meta-analysis by Stamp and Hadfield (2020). The authors showed that for traits that show co-gradient variation (i.e. where plastic and genetic effects are aligned), the plastic responses are in general twice as important. Hence, our plasticity changes are lower compared with many other studies. There could be several reasons for this. Theory predicts that if the costs of plasticity are high and if reliable environmental cues are absent there should be low plasticity (Auld et al., 2010). We suggest that this explanation is unlikely in our study system because in general costs of plasticity have been found to be low or absent (Van Buskirk & Steiner, 2009) and reliable cues, such as temperature, photoperiod or population density should be ubiquitous in these populations. An alternative explanation might be that landmarks are constrained in plasticity since they are not independent, that is they are canalized. Since wings are important for fitness traits such as survival and mating success (Outomuro et al., 2016), then one would expect a high canalization and thus a low plasticity

(Waddington, 1942, 1959). The modest plasticity together with plasticity and genetic differentiation in opposite directions could suggest canalizing selection which have evolved through genetic compensation/counter-gradient variation (Conover & Schultz, 1995; Grether, 2005). A negative correlation would be expected if ancestral plasticity is maladaptive, that is opposes the direction of selection. In this case, we expect more genetic differentiation as it needs to compensate for the maladaptive plastic response that places the organism further away from the adaptive peak. However, this prediction also assumes that the ancestral plasticity cannot evolve. Given that northern and southern environments might differ in how selection acts on wing shape, one would still expect a genetic divergence over time despite a relatively high degree of canalization resulting from purifying selection within each population.

We note that our explanatory power is limited since the r^2 values of our statistical models were quite low. However, comparable r^2 values have been found in other multivariate studies testing for thermal plasticity, genetic differentiation and latitudinal clines of wing shape (Baur et al., 2019; Outomuro et al., 2021; Pitchers et al., 2013; Rohner & Moczek, 2020). Interestingly, we found that families explained a large portion of the variance in wing shape. This suggests a high potential for adaptation in wing shape in this species. We have no good explanation for the high family variation observed. One reason could be that the optimal wing shape differs between populations within northern and southern latitudes. For example, if population density and predation risk differ between years and sites within a region, different wing shapes might be selected between year or sites. Another possibility are common environment effects. Although we reared full-sib families under standard environmental conditions, we cannot account for maternal effects and/or other forms of related trans-generational plasticity. The family effects may thus also include non-additive genetic variation.

5 | CONCLUSIONS

The alignment between plasticity and genetic differences favoured by selection has major impacts on the speed and mode of evolution. We find limited evidence for a general and consistent alignment between thermal/photoperiod plasticity and local adaptation in a damselfly where wing shape has been shown to be subject to selection (e.g. Outomuro et al., 2016). This adds to a growing number of studies on insect wings indicating that evolutionary responses across environmental gradients are difficult to predict based on plasticity alone. Our data highlight the role of genetic differences in plasticity across populations (in terms of direction and magnitude) as important contributors to clinal variation.

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CONFLICT OF INTEREST

We declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The partial warps used for the wing shape analyses used in this article are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.12jm63z20>

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SUPPORTING INFORMATION

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

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