Wing shape evolution is not constrained by ancestral genetic covariances in the invasive *Drosophila suzukii*

Antoine Fraimout[1†], Stéphane Chantepie[1], Nicolas Navarro[2], Céline Teplitsky[3] and Vincent Debat[1]

Short title: Quantitative genetics of *Drosophila suzukii* wing shape

[1]Institut de Systématique, Evolution, Biodiversité, ISYEB – CNRS, MNHN, UPMC, EPHE, Université des Antilles, Muséum National d'Histoire Naturelle, Sorbonne Universités, 57 rue Cuvier, 75005 Paris, France
[2]UMR CNRS 6282 Biogéosciences, Université de Bourgogne-Franche-Comté, 21000 Dijon, France
[3]Centre d'Ecologie Fonctionnelle et Evolutive, CEFE – UMR 5175 – CNRS, IRD, EPHE, Université de Montpellier, Université Paul Valéry, 34293 Montpellier, France

[†]Corresponding author, E-mail: fraimout.antoine@gmail.com

Abstract

The extent to which phenotypic evolution can be constrained by genetic correlations is an important question in evolutionary biology. To address this question, biological invasions are opportune models where derived, invasive populations can be compared to their extant ancestors, allowing to track the evolution of genetic correlations from the ancestor, throughout the invasion process. In this paper, we focused on the worldwide invasion of *Drosophila suzukii* (Matsumara, 1931), and investigated the evolution of the genetic covariance matrix **G** of wing shape between ancestral native, and derived invasive populations. Leveraging demographic history resolved by population genetics approaches, we tested whether **G** remained stable during the invasion. Using a multivariate Q_{ST}-F_{ST} approach, we further tested whether or not the observed phenotypic divergence in wing shape aligned with a neutral scenario of evolution. Our results show moderate yet significant quantitative genetic differentiation of wing shape among *D. suzukii* populations and a relative stability in the structure of **G**, presenting a roughly spherical shape but slightly different volumes. These characteristics likely reflect the demographic history of populations and suggest a low level of genetic constraint on wing shape evolution. The divergence between populations was greater than expected under a purely neutral model of evolution, compatible with an effect of divergent selection among them. Overall, our study suggests that selection, drift but not ancestral genetic constraints affected the early stages of wing shape evolution during *D. suzukii* invasion.

Key words: *Drosophila suzukii*, **G**-matrix, geometric morphometrics, biological invasion, quantitative genetics, Q_{ST}-F_{ST}

Introduction

Biological invasions often involve stochastic demographic changes through founder effects and genetic bottlenecks (Golani *et al.*, 2007) or the shuffling of different alleles by genetic admixture between populations (Rius & Darling, 2014). Invasive populations can also be subjected to multiple and often novel selective pressures in the new environment and throughout the invasion process (Kilkenny & Galloway, 2013). Consequently, they tend to show marked genetic and phenotypic differences compared to their source populations, and distinguishing whether this differentiation results from adaptive or neutral evolution is challenging (Lee, 2002; Wares, 2005; Facon *et al.*, 2006; Hayes & Barry, 2008; Keller & Taylor, 2008; Prentis *et al.*, 2008; Bock *et al.*, 2015; Dlugosch *et al.*, 2015; Estoup *et al.*, 2016). Furthermore, these evolutionary changes can happen over relatively short time scales (Colautti & Lau, 2015). Hence, a topical question in invasion biology is how fast can invasive populations evolve in their new environment? Can we detect adaptive evolution occurring at the onset of the invasion (Szucs *et al.*, 2017)?

In a new environment, the evolution of a population's mean phenotype towards a new fitness optimum (*i.e.* adaptation) is conditioned by the genetic variances and covariances of the traits under selection, summarized in **G**, the genetic variance-covariance matrix (Lande, 1976). For a set of traits measured in a given population, the **G** matrix informs about genetic variation for each individual trait (diagonal elements) and for the genetic covariation between traits (off-diagonal elements). Statistical analysis of **G** through an eigen decomposition can then identify the major axis of variation in the population, corresponding to the combination of traits with the most genetic variation, also known as **g**max (Schluter 1996). Identifying the axes of genetic covariance between traits is particularly important as theory predicts that they will influence the evolutionary response: adaptation will be facilitated if selection is aligned with **g**max, or constrained if it is in a direction where there is little, or no genetic variation for the combination of traits under selection (Arnold *et al.*, 2001, Agrawal & Stinchcombe, 2009; Fischer *et al.*, 2016). This is formalized in the multivariate breeder's equation (Lande, 1979):

$$R = G\beta$$

where the response to selection R - the change in average phenotype of the population in one generation - equals the product of **G** and the directional selection gradient β.

It has been proposed in the context of evolutionary diversification, that the **G** matrix of an ancestral population can constrain derived populations to diverge along certain directions of the phenotypic space (*i.e.* the lines of least evolutionary resistance; Schluter, 1996). For instance, early stages of adaptive phenotypic divergence could be constrained by the direction of the major axis of the ancestral **G** matrix (*i.e.* ancestral **g**max; Schluter, 1996; McGuigan, 2006; Walter *et al.*, 2018) and result in an alignment between **g**max and the leading vector of divergence of the among-populations divergence matrix (**D**). On the other hand, under a neutral model of phenotypic evolution, divergence among populations is also expected to take place along that same axis of greatest genetic variation (Lande 1979) and result in proportional **D** and **G** matrices (Chenoweth *et al.* 2010). In other words, a concordance between the major axes of **D** and **G** could be both suggestive of neutral evolution (Arnold *et al.* 2001; Bégin & Roff, 2003) or indicative of selection.

Furthermore, valid predictions of evolutionary trajectories depend on whether or not **G** remains stable across space and time (Jones *et al.*, 2004; Arnold *et al.*, 2008). The stability of **G** has received much attention (*e.g.* Roff, 2000; Steppan *et al.*, 2002; Bégin & Roff, 2003; Mezey & Houle, 2003; Björklund, 2004; Blows & Hoffmann, 2005; Arnold *et al.*, 2008; Walsh & Blows, 2009) and its sensitivity to selection, migration and drift has been investigated both theoretically (*e.g.* Jones *et al.*, 2004; Guillaume & Whitlock, 2007, Chantepie & Chevin, 2020), and empirically under natural and laboratory conditions (Merilä *et al.*, 1994; Phillips *et al.*, 2001; Conner *et al.*, 2003; Cano *et al.*, 2004; Eroukhmanoff. & Svensson, 2011; Bjorklund *et al.*, 2013; McGlothlin *et al.*, 2018; Walter 2023; Henry & Stinchcombe 2023; Chantepie *et al.* 2024). Multiple lines of evidence suggest that the structure of **G** can be altered by selection (Jones *et al.*, 2003; Jones *et al.*, 2004; Dugand *et al.* 2021), drift (Phillips *et al.*, 2001) and environmental conditions (*e.g.* Sikkink *et al.*, 2015; Wood & Brodie, 2015) even over the span of a single generation (Siren *et al.*, 2017), but, there is also recent evidence for stable **G** matrices across populations and continents (*e.g.*, Puentes *et al.*, 2016; Delahaie *et al.*, 2017, Henry & Stinchcombe 2023). Given the uncertainties around the stability of **G** in specific evolutionary contexts, clear-cut predictions on the influence of **G** on phenotypic evolution in response to selection or drift are difficult to formulate, particularly at a contemporary timescale. Therefore, the stability of **G** needs to be further explored empirically along with clear testing of the neutral expectation for among-population divergence against

observed data (Merilä & Björklund 2004).

Here, we focus on the worldwide invasion of the spotted wing drosophila, *Drosophila suzukii* (Matsumura, 1931) to assess the stability of **G** between native and invasive populations across continents. *Drosophila suzukii* is a highly invasive pest of agricultural crops in Europe and North America where it was first detected almost simultaneously in 2008 (see Asplen *et al.*, 2015 for review). We reconstructed the detailed history of this worldwide invasion and showed the presence of multiple independent introductions from Asia involving population bottlenecks and genetic admixture events within invaded regions (Fraimout *et al.*, 2017). A consequence of this demographic history is that native and invasive populations show significant levels of neutral genetic differentiation as measured by F_{ST} estimated using microsatellite loci (Fraimout *et al.*, 2015; Fraimout *et al.*, 2017). At the phenotypic level, we showed using laboratory lines derived from samples of natural populations that native (Japanese) and invasive (French and North-American) *D. suzukii* display genetically based differences in wing shape and size (Fraimout *et al.*, 2018). However, whether this phenotypic divergence is pervasive across replicated populations, and whether it is associated with changes in the structure of the underlying **G** matrix is unknown. This invasion therefore provides an opportune context to address long-standing questions about the evolution of multivariate phenotypes and the structure of genetic variances and covariances in colonizing populations. Particularly the presence of multiple invasive populations within Europe and USA allows assessing the magnitude and direction of phenotypic evolution from the native ancestral populations. In other words, it is possible to estimate how much the mean phenotype of each invasive population has diverged from the ancestral (*i.e.* native) population and whether phenotypic evolution repeatedly occurred in the same direction or at random, in all directions of the phenotypic space. Given the availability of extant ancestral populations, this in turns allows to determine whether phenotypic divergence among invasive populations has been constrained by ancestral genetic covariances. Furthermore, comparing populations with known history could help identify the effects of demographic processes on the structure of the **G** matrix. For instance, we expect that periods of bottlenecks or population admixture – all experienced by invasive *D. suzukii* populations to a different extent (Fraimout *et al.*, 2017, Olazcuaga *et al.* 2020) – may have altered the structure of **G** in invasive populations. Specifically, depletion of genetic variance due to bottlenecks could result in a **G** matrix of smaller volume. Alternatively, new combinations of alleles produced by genetic

admixture, could increase the volume or **G**, or alter its shape, by changing the distribution of genetic variance over the first axes of variation. Our main objective was thus to investigate whether or not **G** remained stable throughout the invasion history of *D. suzukii*. Additionally, access to both molecular and quantitative measures of genetic variation allows testing for a signal of selection in the observed phenotypic divergence. By comparing F_{ST} calculated from molecular markers to its phenotypic equivalent Q_{ST}, it is possible to determine whether phenotypic divergence exceeds or is equal to the divergence expected under genetic drift alone (Spitze 1993, Whitlock 2008). While the former ($Q_{ST} > F_{ST}$) would be indicative of divergent selection acting on the study traits, the latter ($Q_{ST} = F_{ST}$) would suggest neutral phenotypic evolution.

We used an isofemale line breeding design (Hoffmann & Parsons, 1988; David *et al.*, 2005; Berger *et al.*, 2013) and a geometric morphometric approach (*e.g.* Klingenberg 2003) to estimate the quantitative genetic divergence in wing shape between six natural populations of *D. suzukii* sampled in the native area (Japan) and the two main invaded areas in Europe and the USA. We specifically addressed the following questions: i) how did wing shape evolve across multiple invasive populations? ii) did the **G**-matrix remain stable during the invasion ? iii) has phenotypic divergence among invasive populations been constrained by ancestral genetic covariances and iv) can it be attributed to selection acting on wing shape?

Material and methods

Samples, lines and data acquisition

Drosophila suzukii adults were sampled in 2014 using banana bait traps and net swiping at six localities representative of their global distribution range in Japan (native range), USA and France (invasive range) (Fig. 1). Within each country, we sampled two localities representative of a northern and a southern latitude. Repeated clinal evolution of wing morphology has been described in the related invasive species *Drosophila subobscura* and proposed as an adaption to different temperature optima at northern and southern latitudes (Huey *et al.*, 2000; Gilchrist *et al.*, 2001; Gilchrist *et al.*, 2004). Thus, we hypothesized that sampling populations close to the extremes of their latitudinal distribution would maximize our chance to capture phenotypic and possibly, adaptive, divergence in the invaded range. In USA and France, these localities were also representative of the invasion history with the southern locality corresponding to the first reported

occurrence of *D. suzukii* (Watsonville, USA and Montpellier, France; Hauser, 2011; Calabria *et al.*, 2012) and the northern locality representing the expansion front of the species in the area at the time of sampling (Dayton, Oregon, USA and Paris, France). It is worth stressing that although the most likely genetic source populations of Europe and USA invasions were identified in continental Asia (*i.e.* China, Fraimout *et al.*, 2017), *F* statistics and Bayesian clustering methods indicate weak genetic differentiation between these sources and our Japanese population samples, thereby suggesting that the use of Japanese populations as ancestral representatives is an acceptable approximation. For the Japanese flies, a northern population was sampled in Sapporo (Hokkaido island) and a southern population was sampled in Tokyo (Honshu island). In both Japanese localities, traps were placed in gardens inside the university campuses. In the invasive North American range, sampling habitats corresponded to vineyards in Dayton, and strawberry fields in Watsonville. French samples were collected in the botanical garden of the Natural History Museum in Paris and close to vineyards in Montpellier. An average of 30 isofemale lines (see Table S1 for details and population codes) were established for each locality by placing randomly chosen wild-caught gravid females in separate rearing vials, and splitting F_1 offsprings in five replicate vials. Following the isofemale approach, we further expanded each line by letting individuals mate freely within tubes (*i.e.*, full-sib mating) and transferring their progeny to new rearing vials each generation. We repeated this for five generations and in the fifth generation, a single female per line was isolated in a new rearing tube to produce the focal offspring used in the study. At the end of the experiment, 5 to 10 full-sib males were randomly chosen from each line from each population for phenotyping. Rearing medium consisted of cornstarch and yeast with added anti-biotic (hydroxy-4 benzoate). Lines were randomly positioned in incubators and kept for the five generations at 22°C. A total of 1323 individuals distributed across 177 isofemale lines were phenotyped. Wings were carefully clipped from over-anesthetized flies using tweezers and mounted on glass slides in a mixture of ethanol and glycerin. Coverslips were sealed using nail polish and maintained with small weights to flatten the wing as much as possible. Images of the wings were acquired with a Leica DFC 420 digital camera mounted on a Leica Z6 APO microscope. Fifteen landmarks were defined on the dorsal side of the right wing and digitized using a custom *ImageJ* plugin written by C. P. Klingenberg (pers. comm.).

Wing shape differentiation – Geometric morphometric analyses

176 We focused our study on the analysis of wing shape variation among *D. suzukii* populations. Shape data have
177 been used in multiple quantitative genetics studies (Klingenberg & Leamy, 2001; Klingenberg, 2003; Monteiro
178 *et al.*, 2003; Hansen *et al.*, 2004; Mezey & Houle, 2005; Myers *et al.*, 2006; Polly, 2008; Klingenberg, 2010;
179 Adams, 2011; Leinonen *et al.*, 2011; Martínez-Abadías *et al.*, 2012) and are suitable for the estimation of **G**,
180 and relevant for the study of multivariate phenotypic evolution. We followed a standard geometric
181 morphometrics protocol (Bookstein 1996) to characterize wing shape: first, Procrustes generalized least
182 squares superimposition was used to extract shape information from the landmarks coordinates (GLS, *e.g.*
183 Dryden & Mardia, 1993). Briefly, this method allows to scale, rotate and translate the configuration of
184 landmarks to obtain shape information that is free of orientation or size differences. Second, a principal
185 component analysis (PCA) was applied to the set of 30 coordinates (15 landmarks in 2 dimensions x and y)
186 obtained after superimposition. From the PCA, 26 non-null components were conserved as input data
187 (corresponding to the 30 coordinates minus 4 degrees of freedom lost through the scaling, rotation and
188 translations (x and y)). We estimated the centroid size of the wing as the square root of the sum of the squared
189 distances of all landmarks from their centroid, and used this centroid size as a size variable in the subsequent
190 analyses. As divergence in wing shape first appeared to be weak on the PCA plot, we further performed a
191 between-group principal component analysis (*bgPCA*) to visualize the overall variation in wing shape among
192 populations (*e.g.* Mitteroecker & Bookstein, 2011). We then estimated the statistical significance of the shape
193 divergence among populations by applying a multivariate analysis of variance (MANOVA) using the 26 PCs
194 as response variable, and population of origin as a fixed effect. We further estimated the pairwise differences
195 among populations with permutational pairwise MANOVAs, using the *RVAideMemoire* R package (v.0.9-55,
196 Hervé, 2016). We used the Wilk's lambda statistic and set the number of permutations to 10000 while
197 correcting for multiple testing using the *fdr* method (Benjamini & Hochberg 1995). Finally, to account for any
198 allometric effect - a change in shape associated with a change in size (e.g. Klingenberg 2016), we also fitted
199 the MANOVAs using the residuals of the size/shape regressions, corresponding to the allometry-free
200 components of wing shape variation.
201
202 *Wing shape differentiation – phenotypic divergence vector analysis*
203 To estimate the divergence in wing shape among populations of *D. suzukii*, particularly the magnitude and

direction of phenotypic evolution in the derived invasive populations from their Asian ancestors, we applied a geometric approach to phenotypic evolution (*e.g.,* Stuart *et al.* 2017, Fraimout *et al.* 2022), by calculating vectors of phenotypic divergence among populations. The phenotypic mean of each population was first estimated from the MANOVA (see section above) using the 26 non-null PC representative of wing shape, and accounting for differences in wing size by adding the wing centroid size as covariate. Then, we estimated the differences in mean wing shape between each invasive population and the ancestral wing shape, assessed as the average of the two Japanese populations. We also estimated divergence at the country level, using the three countries average wing shapes. In total, we obtained six phenotypic divergence vectors describing the difference between the ancestral Japanese and each invasive population (4 vectors) and between the ancestor and each country's mean wing shape (2 vectors). To test whether phenotypic divergence occurred along similar or different directions, we assessed the similarity between these vectors of divergence by comparing their angle to the distribution of angles between 10,000 pairs of random vectors of the same dimensionality (Klingenberg and McIntyre, 1998). Angles between vectors were calculated as:

$$\alpha = \cos^{-1}(\Delta z_{Di}, \Delta z^{T}_{Dj}) \quad [1]$$

where z_D corresponds to a divergence vector of population *i* or *j* from the ancestor. An eigenanalysis of the matrix containing the four population vectors was applied to synthesize the main axes of divergences.

*Matrices estimations – population-specific **G** matrices*

We estimated the six **G** matrices corresponding to the six sampled populations under a Bayesian framework using the R-package *MCMCglmm* (Hadfield, 2010). To this end, we partitioned the phenotypic variance in wing shape for each population using a multivariate animal model (Kruuk, 2004) of the form:

$$y = \sum \quad X\beta + \sum \quad Z\gamma + \varepsilon \quad [2]$$

where y is the multivariate vector of phenotypic values for wing shape corresponding to the 26 non-null principal components (PCs) accounting for the totality of shape variance; β is the vector of fixed effect; γ is the vector of random effects; ε is the vector of residual errors and X and Z the design matrices relating to the fixed and random effects, respectively. We estimated **G** matrices separately for each population using model [2] and appended the matrix of relatedness between individuals to the vector of random effects with the

pedigree option implemented in the *MCMCglmm* function. The pedigree corresponding to our crossing design was coded in R using custom scripts (see *Data availability* statement). In all models, wing centroid size was fitted as a covariate. We used a prior set with variance estimates following a 'broken-stick' model of decrease of variance, appropriate for the use of PC scores representing decreasing proportion of explained variance (Jolliffe, 2002), with null covariances and a degree of belief *nu* = n + 0.002 (where n is the number of traits, Houle & Meyer, 2015). We ran each model for 1 200 000 iterations with a burnin period of 200 000 and a thinning rate of 1000, resulting in 1000 Monte Carlo Markov Chain (MCMC) posterior samples. The convergence of models was checked visually from plots of MCMC chains and posterior distributions as well as autocorrelation level (<0.05 in all models). All analyses were performed in R (v.4.2.2; R Core Team, 2022).

*Test of **G**-matrix stability – Differences in the size and shape of **G***

One of our main objectives was to assess the stability of the **G** matrix in the face of the demographic changes linked to the invasion of *D. suzukii.* According to previous results (Fraimout *et al.* 2017) all invasive *D. suzukii* populations have experienced genetic bottlenecks, and some populations show evidence of genetic admixture from differentiated sources. Our hypothesis was that the variation in total genetic variance due to these demographic events - namely, reduction of genetic variance by bottlenecks or increased genetic variance from admixture - should be reflected in the structure of **G**. To test this hypothesis, we estimated three parameters pertaining to the structure of **G** and performed pairwise comparisons among all six studied populations. Matrix size (*i.e.*, volume), estimating the overall amount of genetic variance for wing shape in the population, was calculated as the sum of the matrix eigenvalues. Matrix shape, estimating whether some directions of wing shape variation present more genetic variance than others, was assessed by its eccentricity (*i.e.* the ratio of the first, to the second eigenvalue; Jones *et al.*, 2003). Matrix shape was also estimated by the difference in the proportion of variance along $\mathbf{g}_{max}$. Significance of metric differences between populations was assessed by estimating the ratio between each population metrics and examining the overlap between the 95% highest posterior density (HPD) of these ratios calculated from the MCMC posterior samples, with zero.

*Test of **G**-matrix stability – Genetic covariance tensor*

We further tested for differences among our set of **G** matrices by following the approach of Hine *et al.* (2009)

259 and Aguirre *et al.* (2014) using a fourth-order genetic covariance tensor analysis (hereafter 'covariance
260 tensors'). Briefly (see details in Hine *et al.* 2009; Aguirre *et al.* 2014), variances and covariances among all
261 elements of the original set of **G** matrices are calculated to construct the fourth-order covariance tensor **Σ** and
262 the second-order (*i.e.* matrix) tensor **S**. Eigenanalysis of the **S**-matrix allows for the construction of
263 eigentensors which represent independent vectors of differences in variance and covariance among **G** matrices.
264 Covariance tensors provide a way to compare more than two genetic covariance matrices and describe
265 independent axes of divergence among them. In other words, covariance tensors allow identifying trait
266 combinations explaining most genetic variation among **G** matrices. We applied the covariance tensor analysis
267 to our set of six **G** matrices using the full 1000 MCMC samples for each matrix and compared the observed
268 divergence to the null expectation obtained from the analyses of random **G** matrices. We used an approach
269 based on Morrissey *et al.* (2019) to simulate the null distribution of **G** matrices. For each population, the
270 breeding values of founders were drawn from a multivariate distribution with zero mean and a variance-
271 covariance matrix equal to the **G**-matrix estimated from this population with the animal model [2]. Breeding
272 values were then randomly shuffled among the founders and the breeding values of all remaining individuals
273 were estimated based on the pedigree of each population. As our breeding design included five generations of
274 inbreeding, we modeled a pedigree to better approximate the actual relatedness of our measured individuals.
275 The breeding values of an individual were drawn from a multivariate normal distribution with a vector of
276 means equal to the mean breeding values of its parents and a deviation equal to the segregation variance (Walsh
277 & Lynch, 2018). For an individual, the segregation variance was estimated as half the product of the
278 randomized **G** matrix of their population (estimated on the new population's founders) and one minus the
279 average inbreeding coefficient of its parents (Walsh & Lynch, 2018, pp.557). The phenotypes were then
280 recomposed by adding the random and fixed effects estimated by the animal model following [2].
281
282 We repeated this sequence for each posterior sample of animal models and resulted in 1000 null datasets for
283 each population. The **G** matrix of null datasets was estimated following eq. [2]. To reduce computational
284 burden, we sampled 200 posterior samples for each null animal model (Morrissey *et al*., 2019) to compute a
285 mean null distribution **G** matrix for each population that accounts for both the sampling effect and the
286 uncertainty in the **G** matrix estimates. Each final null **G** matrix consisted of the average of the 1000 MCMC

posterior for each of the 100 replicates and were used to estimate random genetic covariance tensor as described in Aguirre *et al.* (2014) using custom scripts in R (see *Data availability* section for source code),

*Test of genetic constraints: proportionality between **G** and **D***

In the case of populations deriving from a common ancestor, quantitative genetics theory predicts that genetic constraints imposed by the ancestral **G**-matrix can influence among-population divergence in a manner, leading to the proportionality of the divergence matrix **D** and the ancestral **G** under neutral or adaptive scenarios (Lande, 1979; Merilä & Björklund 1999, 2004). This proportionality induced by genetic constraints is expected under selection, where the evolutionary response is biased along the ancestral main directions of genetic variation (e.g. Schluter 1996, MacGlothlin et al 2018), but also under neutral evolution, as genetic drift is expected to induce a global reduction in genetic variances, but no predictable change in the orientation of the divergence, leaving on average **G** and **D** proportional (Lande *et al*. 1979; Phillips *et al* 2001).

The similarity between **D** and **G** was first investigated by computing the angle between a divergence vector ***d*** and the ancestral **G** matrix following Krzanowski (1979):

$$\theta = cos^{-1}\{(dLL^t d^t)^{-0.5}\}$$

where **L** is the set of first q eigenvectors of **G**, where q was set equal to half the shape space dimensionality ($q = (2k - 4)/2 = 13$; and k is the number of digitized landmarks) and ***d*** is a divergence vector..

We compared the observed angles to their random expectations by simulating 1000 random vectors v_{2k} projected in the tangent space according to

$$d = (I_{2k} - \mu\mu^t)v$$

where $\boldsymbol{\mu}$ is the vectorised mean shape (Dryden and Mardia, 1998).

Test of selection: multivariate Q_{ST}-F_{ST} comparison

A proportionality between **G** and **D** is insufficient to identify the evolutionary process at play, but is indicative of an effect of genetic constraints on phenotypic evolution pointing at the need for a more stringent test to decipher the adaptive and neutral scenarios (Martin *et al*. 2008). To test the hypothesis that phenotypic evolution among invasive *D. suzukii* populations resulted from divergent selection on wing shape, we used the

314 two steps method proposed by Martin *et al.* (2008). Briefly, this method is a multivariate extension of the
315 classical Q_{ST}-F_{ST}, testing whether the observed phenotypic divergence is greater ($Q_{ST} > F_{ST}$), equal to ($Q_{ST} =$
316 F_{ST}), or smaller ($Q_{ST} < F_{ST}$) than expected under drift alone (see Martin et al. 2008 and Chapuis et al. 2008 for
317 more details). First, proportionality between **D** and **G** was tested using the MANOVA estimates of the
318 covariance matrices. Second, the coefficient of proportionality ρ_{ST} between the ancestral **G** matrix and the
319 divergence matrix **D** was estimated and compared to the predicted neutral divergence $2F_{ST}/(1-F_{ST})$ obtained
320 from neutral molecular markers (Rogers and Harpending 1983; Martin et al 2008). Deviation from this value
321 would be indicative of selection. To test for proportionality between **D** and **G**, we used the Bartlett-adjusted
322 test of proportionality between matrices as recommended in Martin *et al.* (2008). We used the *k.prop* R function
323 of the neutrality package from Martin *et al.* (2008) to calculate ρ_{ST} and the *hierfstat* R package (v.0.5.11; Goudet
324 & Jombart 2022) to get bootstrapped estimates of F_{ST} from 25 neutral microsatellite loci (Fraimout *et al.* 2015,
325 2017). Inputs for both tests were obtained by performing a MANOVA on the wing shape data and using the
326 log-centroid size as covariate and population of origin as fixed effect. Also following recommendation from
327 the authors, we used the first 11 PCs describing wing shape variation to maximize accuracy of the statistical
328 tests so that $p < 2nb$ (where p is the number of traits and nb is the number of populations sampled) while
329 retaining PCs accounting for > 90% of variance.
330
331 *Test of selection: solving the multivariate breeder's equation*
332 It has been suggested that in Drosophila, flight performance in different thermal conditions might be under
333 selection, favouring the evolution of latitudinal gradients in wing size and shape (e.g. Gilchrist *et al.* 2000).
334 This hypothesis is indirectly supported by phenotypic plasticity analyses, showing better flight performance in
335 cold air in flies reared at low temperatures, associated with different wing morphologies (Frazier *et al.* 2008).
336 While we are mostly oblivious to the selective factors affecting *D. suzukii* invasive success (but see Camus *et*
337 *al.* 2025), and specifically the evolution of its wing morphology, flight performance might play a role in its
338 dispersive ability, or be under selection in the diverse thermal conditions encountered across its latitudinal
339 ranges. We used data obtained in a previous study showing an association between wing shape and flight speed
340 in *D. suzukii* (Fraimout *et al.* 2018), to explore the hypothesis that selection may have been involved in the
341 divergence of wing shapes among invasive populations, using the multivariate breeder's equation (Lande

342 1979). First, an experimental selection differential was derived from Fraimout *et al*. (2018) data. In this
343 previous study, we reconstructed 3D flight trajectories of flies reared under different developmental
344 temperatures and measured flight parameters such as speed, acceleration, or sinuosity. Recorded individual
345 wings were digitized and wing landmarks coordinates used to assess the relationship between flight parameters
346 and wing shape. Landmark data of this previous study were aligned onto the reference configuration of the
347 present study to allow direct comparability. Solving the breeder's equation assumes that traits change is
348 correlated with fitness. In the absence of fitness data, we hypothesized that flight performance might
349 significantly contribute to fitness in Drosophila (e.g. Frazier *et al*. 2008) and we therefore used the shape
350 change associated with the strongest flight speed difference as a hypothetical selection differential. Wing shape
351 was regressed onto the log of flight speed using a multivariate regression (*e.g.* Mardia *et al.* 1979; Monteiro
352 1999) to estimate the vector of shape change associated with highest flight velocity s_{velocity}. The corresponding
353 response to selection Δz was then predicted using the multivariate breeder's equation (Lande and Arnold 1983)
354 and the estimated ancestral (*i.e.* Japanese) **G** and **P** matrices:

355 $$\Delta z = G_{jap} P_{jap}^{-} s_{velocity}$$

356 where $^{-}$ is the Moore-Penrose generalized inverse. This predicted response to velocity selection was compared
357 to the different vectors of population divergences (see above) by computing the angle between them following

358 $$\alpha = cos^{-1} \frac{\Delta z}{\|\Delta z\|} \cdot \frac{d^t}{\|d\|}$$

359 The observed angles were then compared to the expectation of the angle between Δz and 1000 random vectors
360 (see above). Our hypothesis here is that if wing shape associated with higher flight speed would be a target of
361 selection, we should observe a match between the evolutionary shape changes (the phenotypic divergence
362 among populations) and the shape change resulting from the hypothetical response to selection.
363

364 *Test of selection: percentage of genetic variance along divergence vectors*

365 Finally, we reasoned that if selection was involved in the short-term evolution of wing shape, it should manifest
366 in a depletion of genetic variation in the selected directions (*e.g.* Agrawal and Stinchcombe 2009),
367 corresponding to the directions of divergence from the hypothetical common ancestor. To test this prediction,

368 **G** matrices of each population were projected onto the shape space directions defined by the divergence
369 vectors, $d^t Gd$, and expressed in proportion of the total genetic variance. The amount of genetic variance in
370 the direction defined by ***d*** was then compared between the ancestral and derived **G** matrices. We predicted that
371 a reduction in this proportion of variance should be detected if selection was involved in the evolutionary
372 divergence, as depletion of genetic variance due to drift would randomly affect all directions. To test whether
373 genetic variance along the directions of divergence was lower than expected by chance, we compared it to the
374 variance estimated in random directions in the (tangent) shape space.
375
376 **Results**
377 *Significant divergence of wing size and shape across populations*
378 We observed differentiation in wing centroid size between American and all other populations, as well as
379 between the two American populations (Fig. S1): the American populations displayed smaller wing size, with
380 US-WAT displaying the smallest wings. Although the divergence for wing shape among populations was weak,
381 the *bgPCA* showed a signal of geographic differentiation, opposing the pairs of populations according to their
382 geographic origin (Fig. S2). This shape divergence was confirmed by the significant population effect in the
383 MANOVA (Wilk's lambda = 0.143, df = 5, $p < 0.001$). The MANOVA performed on the residuals of the
384 regression between shape and size also revealed a significant population effect (Wilk's lambda = 0.199, df =
385 5, $p < 0.001$), suggesting that the shape difference goes beyond a simple allometric effect. The pairwise
386 permutational MANOVAs revealed significant shape differences between all pairs of populations ($p < 1e\text{-}04$).
387 The analysis of the angles between the divergence vectors suggests that both French populations diverged from
388 their common ancestor in the same direction of the phenotypic space: these two vectors are more similar than
389 two random vectors ($\alpha = 31.9°$, $p < 10^{-4}$, Fig. 2, Table S2). For the American populations, the angle between
390 the two divergence vectors was greater than that of the French populations ($\alpha = 49.9°$, $p = 5 \times 10^{-4}$, Fig 2, Table
391 S2) and hint towards divergence along different directions. The average American and French direction of
392 divergence from their common ancestor were strikingly different, and no more similar than random vectors (α
393 $= 71.9°$, $p = 0.11$, Table S2). In terms of amount of divergence based on the Procrustes distance, the American
394 populations appeared to have slightly less diverge than French populations, from their hypothetical ancestor
395 (5.72×10^{-3}, 7.46×10^{-3}, respectively; Table S3) and as observed from the *bgPCA* (Fig. S2).

*The **G**-matrix for wing shape is mostly stable across populations*

We found significant differences in the size of the **G** matrix among 3 pairs of populations, all involving the southern USA population US-WAT (Table 1). The **G** matrix of the US-WAT population had a significantly larger volume than those of US-DAY (northern USA) and of both French populations (FR-PAR and FR-MON). None of the other pairwise comparisons revealed any significant differences in the volumes of **G**.

Overall, all population-specific **G**-matrices appeared relatively spherical (Fig. 3). Eccentricity values calculated as the ratio of the first to the second eigenvalues of **G** were low and close to 1 for all matrices (Fig. S3). In other words, genetic variation in each population was relatively equally distributed among combinations of traits. We did not find any significant differences in the shape of the **G** matrices as eccentricity and the amount of variance explained by $\boldsymbol{g}_{max}$ between any pairs of populations were not statistically different (Fig. S3). Similarly, no significant difference was found between the eigentensors of **S** calculated from the set of random and observed sets of **G** matrices based on the overlapping 95% HPD intervals (Fig. 4), suggesting a global stability of the **G** matrices across populations.

*Proportionality of **D** and ancestral **G**: a constrained response to selection?*

Overall, the divergence matrix appeared fairly similar to the ancestral **G** matrix, its first two PCs and the ancestral **G** matrix respectively forming narrow angles of 13.6 and 15.8°, smaller than expected for random vectors (47.9°± 7.18). While the null hypothesis of proportionality between both matrices was not rejected (Bartlett-adjusted X^2 test; $p = 1$), suggesting a close match, the coefficient of proportionality ($\rho = 1.063$; 95% CI, 0.763 – 1.751) was in turn higher than the coefficient estimated for microsatellite markers ($2F_{ST}/(1-F_{ST})$ = 0.160; 95% CI, 0.128 – 0.198). Therefore, while **D** and **G** are proportional, suggesting a genetic constraint, their coefficient of proportionality deviates from the neutral expectation, suggesting a role for selection in the observed divergence.

No reduction in genetic variance in the direction of divergence

We further projected each **G** matrix onto their respective direction of divergence from the hypothetical ancestor and estimated the corresponding percentage of variance (Fig. 5). Overall, we found that amounts of genetic

variance in these directions were not statistically different among populations. Furthermore, we did not observe a lower amount of genetic variance between the projection of each population **G** matrix and the projection of the ancestral **G** matrix on their corresponding divergence vectors. This result goes against our expectation that selection would result in a depletion of genetic variance along each population's divergence vector from the ancestor.

The response to a theoretical selection does not match the observed divergences

To investigate the possible selective factors affecting wing shape evolution in *D. suzukii* invasion, we simulated the evolutionary responses to a hypothetical selection differential, the wing shape difference between fast and slow flying *D. suzukii* individuals, as reported in Fraimout *et al.* (2018; Fig. 6a). The evolutionary response is shown on Fig. 6b, as the average response derived from the full posterior of MCMC samples. The shape divergence of French and American populations from their common hypothetical ancestor are shown on Figure 6c and d (the two Japanese populations used to compute the ancestral **G** matrix were considered too close from this ancestral state and were thus not considered). The pattern of shape change associated with $\boldsymbol{S}_{velocity}$ and the average response Δz were very similar (mean α = 22.74°, SD 6.03) confirming that **G** does not constrain the response to selection. This result is in line with the relative spherical shape of the ancestral **G** matrix, which should not constrain the response to selection. While a similarity in the shift of the basal landmarks can be found between the divergence of French populations and the theoretical response to selection, no obvious similarity with American populations divergences is detected (Fig 6b,c,d, Fig. S4). However, this congruence was not reflected in the correlation between vectors when accounting for the full landmark configurations as the evolution of the distal part of the wing did not match the expected response to selection (Fig. 6b, c, Fig. S4). Overall, the similarity between the divergence vectors from each population and the response to selection Δz was low (Fig. S4) suggesting that the observed divergence in wing shape does not result from selection on faster flight capacity.

Discussion

Genetic divergence of wing shape across populations of Drosophila suzukii

Geographical variation of wing morphology has been described in several *Drosophila* species (*e.g.* Azevedo

452 *et al.*, 1998; Haas & Tolley, 1998; Hoffmann & Shirriffs, 2002), including introduced ones (Huey *et al.*, 2000).
453 In particular, geographical clines of wing size and shape have been detected along latitudinal and altitudinal
454 gradients (Huey *et al.* 2000, Gilchrist *et al.* 2004; Gilchrist *et al.*, 2001; Gilchrist *et al.*, 2004; Pitchers *et al.*,
455 2013; Adrion *et al.*, 2015). These clines have typically been interpreted as adaptations to flight conditions,
456 specifically temperature and air pressure (Dillon and Frazier 2006; Pitchers et al 2013), although direct
457 evidence on the nature of selection is lacking. In *D. suzukii*, clines in wing morphology have been reported
458 along an altitudinal gradient in Hawaii – the first and oldest invaded territory (Asplen *et al.*, 2015) – with flies
459 from high altitude (ca. 2000m) displaying significantly larger wings than flies sampled at sea level (Koch *et*
460 *al.*, 2020). Here, we detected geographic variation of wing shape across all populations, but the differences
461 among north and south populations were different among continents. Gilchrist *et al.* (2000) reported
462 differences in latitudinal wing shape changes in *D. melanogaster* from three continents, and suggested the
463 occurrence of specific local selective pressures on wing shape. However, they also detected parallel clines in
464 wing size across continents, suggesting common adaptive effects. In our study, wing size was more stable: the
465 only difference between northern and southern populations was found in the USA. As for wing shape, although
466 we found that populations from the same continent have evolved in a relatively similar direction of the
467 phenotypic space, we did not find any evidence for parallel latitudinal clines. At the time of sampling, the
468 invasion of *D. suzukii* was initiated less than a decade ago in Europe and the USA and it is possible that adaptive
469 geographical clines did not have enough time to evolve in these regions, contrary to the first invaded range in
470 Hawaii (Koch *et al.* 2020). As a comparison, a latitudinal cline in *D. subobscura* following colonization of the
471 New World, was reported after two decades, while no difference had been detected ten years after the start of
472 the invasion (Huey *et al.*, 2000; Gilchrist *et al.*, 2004). At this point, it is unclear whether *D. suzukii* wing
473 phenotypes are locally adapted, and additional work is warranted to decipher the interplay between wing
474 plasticity, genetics and their association with fitness in natural *D. suzukii* populations. Nevertheless, by rearing
475 flies in controlled laboratory conditions for a few generations, we showed that the phenotypic divergence
476 observed among populations is genetically based rather than plastically induced (see also Fraimout *et al* 2018)
477 and that some evolutionary process independent of phenotypic plasticity have shaped the wings of *D. suzukii*
478 in the wild.
479

480 ***G** matrix of wing shape is stable across populations*

481 Despite the wealth of theoretical and empirical research (e.g. Roff, 2000; Steppan *et al.*, 2002; Bégin & Roff,
482 2003; Jones *et al.*, 2003; Mezey & Houle, 2003; Björklund, 2004; Jones *et al.*, 2004; Blows & Hoffmann,
483 2005; Arnold *et al.*, 2008; Walsh & Blows, 2009; Jones *et al.*, 2014; Wood & Brodie, 2015; Puentes *et al.*,
484 2016; Svensson *et al.*, 2021), predicting how natural selection or drift will affect the structure of **G** and at
485 which pace **G** can evolve in the wild remains a difficult task. As stated by Jones *et al.* (2003): "[...] *the current*
486 *state of analytical theory on **G**-matrix evolution is that existing theory cannot guarantee stability of the **G**-*
487 *matrix (Turelli 1988), but also does not guarantee instability*." Following this assertion, simulation studies
488 (Jones *et al.*, 2003; Jones *et al.*, 2004; Arnold *et al.*, 2008; Jones *et al.*, 2014; Björklund & Gustafsson, 2015;
489 Chantepie & Chevin 2020) have identified how correlative selection, pleiotropic mutations, population size
490 and other evolutionary forces can affect the stability of **G**'s structure and orientation. However, empirically
491 and 20 years later, evidence for both stability (Delahaie *et al.*, 2017; McGlothlin *et al.*, 2018, Henry *et al.* 2023)
492 and lability (*e.g.*, Uesugi *et al.*, 2017, Chantepie et al. 2024) have been reported in varying contexts.

493 Here we found that the **G** matrices were globally stable across populations, the differences between populations
494 mainly pertaining to the volume of **G** (i.e. the global amount of genetic variance). Specifically, French
495 populations seemed to display less genetic variance as indicated by their smaller volumes, while the US-WAT
496 population seemed more genetically variable than the rest of the populations. Reduction of genetic variance
497 and, therefore, of the volume of **G**, is expected under certain conditions such as increased genetic drift due to
498 reduced effective population size (Shaw *et al.*, 1995; Roff, 2000; Jones *et al.*, 2004) or increased strength of
499 stabilizing selection (Jones *et al.* 2003). According to the results of Fraimout *et al.* (2017) obtained from neutral
500 molecular markers and Olazcuaga *et al.* (2020) using whole-genome data, all invasive populations of *D. suzukii*
501 experienced significant loss of genetic diversity due to various intensities of bottlenecks upon introduction.
502 Lesser levels of genetic variance and lower volumes in the French **G** matrices are thus consistent with the
503 effect of drift. Similarly, the higher volume of the US-WAT population could be explained by the genetic
504 admixture of two highly differentiated lineages from Asia and Hawaii (Fraimout *et al.* 2017). However,
505 populations' invasion history was not reflected in all **G** matrices, as other genetically admixed populations
506 (FR-PAR, US-DAY; Fraimout *et al.* 2017) did not show higher volumes and neither did ancestral populations
507 where genetic bottlenecks most likely did not occur. Contrasting with the random evolution of **G** in all

508 directions of the multivariate space expected under genetic drift (Arnold *et al.*, 2001; Phillips *et al.*, 2001), our
509 results show that most aspects of the eigenstructure of **G** (size and shape) have remained stable throughout the
510 invasion process. The observed stability of **G** among populations is also consistent with strong stabilizing
511 selection acting on wing shape (Pélabon *et al.* 2004). In a recent study, Bränden & De Lisle (2026) have shown
512 that stabilizing selection is pervasive in Drosophila, and constitutes a significant constraint on wing shape
513 evolution. Without access to fitness assays and its relationship to wing shape variance in our study, this
514 hypothesis cannot be formally tested with the data at hand. Further work on *D. suzukii* would thus be valuable
515 to shed light on the effect of stabilizing selection on the stability of the wing shape **G** matrix.
516
517 Our analyses may lack statistical power to detect more subtle differences between **G** matrices. Indeed, while
518 our crossing design consists of an acceptable number of genetic lines per population, the multidimensional
519 nature of the *Drosophila* wing implies a large number of parameters to be estimated (n x (n-1) /2 + n; 351 in
520 our case), larger than the number of replicates (*i.e.* isofemale lines). Nonetheless, replicating our analyses using
521 fewer numbers of PCs - and thus less parameters to be estimated - led to the same conclusions (see
522 *Supplementary material*). Finally, our design did not allow us to partition additive from non-additive genetic
523 components of wing shape variation. Dominance genetic variance has been shown to explain up to 35% of
524 phenotypic variation for wing traits in *D. serrata* (Sztepanacz & Blows 2015) and it is therefore possible that
525 some of our estimates of genetic variance could be inflated by such non-additive components. Whether
526 between-population differences exist in the importance of dominance variance for wing shape is unknown, and
527 future work including appropriate breeding designs (*e.g.,* double-cousins) should shed light on the importance
528 of non-additive genetic components in wing shape variation of *D. suzukii*.
529
530 *Phenotypic divergence is not constrained in D. suzukii's invasive populations*
531 We tested the hypotheses of ancestral constraints on wing shape evolution by estimating the similarity between
532 the ancestral **G** matrix and the divergence matrix **D**, and found that the leading vectors of both matrices were
533 tightly associated. Such similarity can be viewed as a mark of genetic constraints affecting divergence,
534 compatible with both neutral and adaptive scenarios. Genetic drift is expected to induce divergence along the
535 axes with the most genetic variation, and proportionally to the amount of variation in the ancestral population

536 (Lande 1979; Lofsvold 1998; Marroig and Cheverud, 2004). But this pattern of concordance between **G** and
537 **D** is also expected under selection, the main directions of genetic variation biasing the response to selection
538 (i.e. the lines of genetic least resistance; Schluter, 1996). However, the estimated ancestral **G** matrix for *D.*
539 *suzukii* wing shape had a relatively spherical shape, suggesting the existence of genetic variation in all
540 directions of the phenotypic space, and therefore a lack of genetic constraint. This is evident from the results
541 of our phenotypic divergence vector analysis showing that, despite the relatively small differentiation among
542 populations, the evolution of wing shape in France and USA has followed very different directions, suggesting
543 no constraint. Finally, the response to selection predicted from our theoretical selection gradient applied to the
544 estimated ancestral **G** produced a wing shape different from that observed in any of the extant invasive
545 populations, further supporting an absence of genetic constraint on wing shape evolution in this species.
546
547 *Adaptive or neutral divergence?*
548 The results from our multivariate Q_{ST}-F_{ST} support an adaptive scenario for wing shape evolution, as divergence
549 was found to be significantly greater than expected under neutral evolution. While failure to account for
550 demographic history and population structure can bias the outcome of Q_{ST}-F_{ST} comparisons (de Villemereuil
551 *et al.* 2020, do O *et al.* 2025), it does not seem to be the case here. The demographic history of *D. suzukii* is
552 known, and there is no evidence for gene flow or migration among populations (even within-country; Fraimout
553 *et al.* 2017) that could reduce values of F_{ST}, biasing upward the result for our test of selection ($\rho st > F_{ST}$). As
554 for a possible inflation of ρst, this could happen if all populations had undergone drastic bottlenecks and
555 reductions in **G** while **D** remained large. We do not have evidence that this happened in our study populations,
556 as the volume of most invasive populations' **G** is not significantly smaller than that of the native population.
557 The divergence in wing shape among invasive populations of *D. suzukii* might thus be adaptive. The statistical
558 power of this test is conditional on the sampling design and particularly the number of populations sampled
559 (Martin *et al.* 2008). While the test for departure of neutrality should be robust to a small number of populations
560 (*i.e.,* < 10 as in the current study) results of the proportionality test should be interpreted with caution. Similar
561 analyses using long-diverged groups (*i.e.,* Teosinte and Maize), report values of ρ_{ST} several order of magnitude
562 above the neutral expectation (Yang *et al.* 2019), suggesting that the signal of selection we observe in *D. suzukii*
563 is rather low. The adaptive value of wing shape variation in *Drosophila* is a long-standing conundrum, and in

the absence of fitness data, remains speculative (Gilchrist *et al.* 2000). Given the role of wing in flight, we leveraged available flight data (Fraimout *et al* 2018) to test whether selection on flight speed could be involved in wing shape divergence among our populations. Despite partial correspondence, the response to this selection applied to the ancestral **G** matrix, was very different from the observed evolutionary divergence, providing no support for selection on flight speed as a driver of this divergence. The nature of the selective factors affecting wing shape evolution thus remains to be identified.

Conclusion

Consensual predictions on the effect of selection and drift on the **G** matrix remain scarce in the literature and most empirical evidence are based on experimental evolution studies (*e.g.* Doroszuk *et al.*, 2008; Careau *et al.*, 2015) or comparisons among long-diverged species (e.g. McGlothlin *et al.*, 2018). In this context our study provides new insights on the evolution (or lack thereof) of **G** over a relatively short evolutionary time scale in natural conditions. We showed that native and invasive populations of *D. suzukii* display quantitative genetic differentiation in wing shape across continents and at an early stage of their worldwide invasion. Our results suggest an effect of selection acting on a poorly constraining ancestral G matrix. They provide a reference point for future studies quantifying phenotypic evolution in this species.

Acknowledgments

AF was supported by the Agence Nationale de la Recherche, through the LabEx ANR-10-LABX-0003-BCDiv, of the program "Investissements d'avenir" (ANR-11-IDEX-0004-02). VD was funded by ANR SWING (ANR-16-CE02-0015). The authors are grateful to S. Fellous, A. Xuéreb, K. Tamura, M. Toda, P. Shearer, T. Schlenke and A. Kopp for help with flies sampling. We thank M. Guillaume for helping with the stock flies maintenance and F. Peronnet for providing flies rearing medium.

Author contribution

AF, VD and CT designed the study; AF, SC and NN analyzed the data; AF and VD obtained the samples; AF led the writing of the first draft and all authors contributed critically to the manuscript.

Data availability

Data and code necessary to replicate the analyses presented in the manuscript have been deposited on the Zenodo platform (zenodo.org).

References

Adams, D. C., & Otárola-Castillo, E. (2013). geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in ecology and evolution*, *4*(4), 393-399.

Adams, D. C. (2011). Quantitative genetics and evolution of head shape in Plethodon salamanders. *Evolutionary Biology*, *38*(3), 278-286.

Adrion, J. R., Hahn, M. W., & Cooper, B. S. (2015). Revisiting classic clines in Drosophila melanogaster in the age of genomics. *Trends in Genetics, 31*(8), 434-444.

Agrawal, A. F., & Stinchcombe, J. R. (2009). How much do genetic covariances alter the rate of adaptation? *Proc Biol Sci, 276*(1659), 1183-1191.

Arnold, S. J., Burger, R., Hohenlohe, P. A., Ajie, B. C., & Jones, A. G. (2008). Understanding the evolution and stability of the G-matrix. *Evolution, 62*(10), 2451-2461.

Arnold, S. J., Pfrender, M. E., & Jones, A. G. (2001). The adaptive landscape as a conceptual bridge between micro-and macroevolution *Microevolution Rate, Pattern, Process* (pp. 9-32): Springer.

Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., Gibert, P., Gutierrez, A. P., Hoelmer, K. A., Hutchison, W. D.*, et al.* (2015). Invasion biology of spotted wing Drosophila (Drosophila suzukii): a global perspective and future priorities. *Journal of Pest Science, 88*(3), 469-494.

Azevedo, R. B., James, A. C., McCabe, J., & Partridge, L. (1998). Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in Drosophila melanogaster. *Evolution*, *52*(5), 1353-1362.

Bégin, M., & Roff, D. A. (2003). The constancy of the G matrix through species divergence and the effects of quantitative genetic constraints on phenotypic evolution: a case study in crickets. *Evolution*, *57*(5), 1107-1120.

Berger, D., Postma, E., Blanckenhorn, W. U., & Walters, R. J. (2013). Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. *Evolution, 67*(8), 2385-2399.

Björklund, M. (2004). Constancy of the G Matrix in Ecological Time. *Evolution, 58*(6).

Björklund, M., & Gustafsson, L. (2015). The stability of the G-matrix: The role of spatial heterogeneity. *Evolution*, *69*(7), 1953-1958.

Bjorklund, M., Husby, A., & Gustafsson, L. (2013). Rapid and unpredictable changes of the G-matrix in a natural bird population over 25 years. *Journal of Evolutionary Biology, 26*(1), 1-13.

Blows, M. W., & Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology, 86*(6), 1371-1384.

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hubner, S., Turner, K. G.Whitney, K. D., & Rieseberg, L. H. (2015). What we still don't know about invasion genetics. *Molecular Ecology, 24*(9), 2277-2297.

Bränden, A., & De Lisle, S. P. (2026). Apparent stabilizing selection explains micro and macroevolution of Drosophila wings. *bioRxiv*, 2026-01.

Calabria, G., Máca, J., Bächli, G., Serra, L., & Pascual, M. (2012). First records of the potential pest species Drosophila suzukii (Diptera: Drosophilidae) in Europe. *Journal of Applied Entomology, 136*(1-2), 139-147.

Camus, L., Rode, N. O., Serga, S., Loiseau, A., Chen, X., Iampietro, C., ... & Gautier, M. (2025).

Adaptive Challenges of Past and Future Invasion of Drosophila suzukii: Insights From Novel Genomic Resources and Statistical Methods Combining Individual and Pool Sequencing Data. *Molecular Ecology*, e70192.

Cano, J. M., Laurila, A., Palo, J., & Merilä, J. (2004). Population differentiation in G matrix structure due to natural selection in Rana temporaria. *Evolution*, *58*(9), 2013-2020.

Careau, V., Wolak, M. E., Carter, P. A., & Garland, T. (2015). Evolution of the additive genetic variance–covariance matrix under continuous directional selection on a complex behavioural phenotype. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1819).

Chantepie, S., & Chevin, L. M. (2020). How does the strength of selection influence genetic correlations?. *Evolution letters*, *4*(6), 468-478.

Chantepie, S., Charmantier, A., Delahaie, B., Adriaensen, F., Matthysen, E., Visser, M. E., ... & Teplitsky, C. (2024). Divergence in evolutionary potential of life history traits among wild populations is predicted by differences in climatic conditions. *Evolution Letters*, *8*(1), 29-42.

Chenoweth, S. F., Rundle, H. D., & Blows, M. W. (2010). The contribution of selection and genetic constraints to phenotypic divergence. *The American Naturalist*, *175*(2), 186-196.

Colautti, R. I., & Lau, J. A. (2016). Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Invasion Genetics: The Baker and Stebbins Legacy*, 101-121.

Conner, J. K., Franks, R., & Stewart, C. (2003). Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution, 57*(3), 487-495.

Crnokrak, P., & Merilä, J. (2002). Genetic population divergence: markers and traits. *Trends in Ecology & Evolution*, *17*(11), 501.

David, J. R., Gibert, P., Legout, H., Pétavy, G., Capy, P., & Moreteau, B. (2005). Isofemale lines in Drosophila: an empirical approach to quantitative trait analysis in natural populations. *Heredity*, *94*(1), 3-12.

Delahaie, B., Charmantier, A., Chantepie, S., Garant, D., Porlier, M., & Teplitsky, C. (2017). Conserved G-matrices of morphological and life-history traits among continental and island blue tit populations. *Heredity*, *119*(2), 76-87.

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., & Gillette, H. D. (2015). The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology, 24*(9), 2095-2111.

Doroszuk, A., Wojewodzic, M. W., Gort, G., & Kammenga, J. E. (2008). Rapid divergence of genetic variance-covariance matrix within a natural population. *The American Naturalist, 171*(3), 291-304.

Dryden, I. L., & Mardia, K. V. (1993). Multivariate shape analysis. *Sankhyā: The Indian Journal of Statistics, Series A*, 460-480.

Dugand, R. J., Aguirre, J. D., Hine, E., Blows, M. W., & McGuigan, K. (2021). The contribution of mutation and selection to multivariate quantitative genetic variance in an outbred population of Drosophila serrata. *Proceedings of the National Academy of Sciences*, *118*(31).

Eroukhmanoff., & Svensson, E. I. (2011). Evolution and stability of the G-matrix during the colonization of a novel environment. *Journal of Evolutionary Biology, 24*(6), 1363-1373.

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is There a Genetic Paradox of Biological Invasion? *Annual Review of Ecology, Evolution, and Systematics, 47*(1), 51-72.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A., & David, P. (2006). A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution, 21*(3), 130-135.

Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016). Plasticity and evolution in correlated suites of traits. *Journal of Evolutionary Biology, 29*(5), 991-1002.

Fraimout, A., Debat, V., Fellous, S., Hufbauer, R. A., Foucaud, J., Pudlo, P., ... & Estoup, A. (2017). Deciphering the routes of invasion of Drosophila suzukii by means of ABC random forest. *Molecular biology and evolution*, *34*(4), 980-996.

Fraimout, A., Jacquemart, P., Villarroel, B., Aponte, D. J., Decamps, T., Herrel, A., ... & Debat, V. (2018). Phenotypic plasticity of Drosophila suzukii wing to developmental temperature: implications for flight. *Journal of Experimental Biology*, *221*(13).

Fraimout, A., Loiseau, A., Price, D. K., Xuereb, A., Jean-Francois, M., Vitalis, R., Fellous, S., Debat, V., & Estoup, A. (2015). New set of microsatellite markers for the spotted-wing Drosophila suzukii (Diptera: Drosophilidae): A promising molecular tool for inferring the invasion history of this major insect pest. *European Journal of Entomology, 112*(4), 855-859.

Fraimout, A., Päiviö, E., & Merilä, J. (2022). Relaxed risk of predation drives parallel evolution of stickleback behavior. *Evolution*, *76*(11), 2712-2723.

Gilchrist, G. W., Huey, R. B., Balanyà, J., Pascual, M., & Serra, L. (2004). A Time Series of Evolution in Action: A Latitudinal Cline in Wing Size in South American Drosophila Subobscura. *Evolution, 58*(4).

Gilchrist, G. W., Huey, R. B., & Serra, L. (2001). Rapid evolution of wing size clines in Drosophila subobscura. *Genetica*, *112*(1), 273-286.

Golani, D., Azzurro, E., Corsini-Foka, M., Falautano, M., Andaloro, F., & Bernardi, G. (2007). Genetic bottlenecks and successful biological invasions: the case of a recent Lessepsian migrant. *Biology letters*, *3*(5), 541-545.

Goudet, J., & Martin, G. (2007). Under neutrality, Q ST≤ F ST when there is dominance in an island model. *Genetics*, *176*(2), 1371-1374.

Guillaume, F., & Whitlock, M. C. (2007). Effects of migration on the genetic covariance matrix. *Evolution*, *61*(10), 2398-2409.

Haas, H. L., & Tolley, K. A. (1998). Geographic variation of wing morphology in three Eurasian populations of the fruit fly, Drosophila lummei. *Journal of Zoology*, *245*(2), 197-203.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software, 33*(2), 1-22.

Hansen, T. F., & Houle, D. (2004). Evolvability, stabilizing selection, and the problem. *Phenotypic integration: Studying the ecology and evolution of complex phenotypes*, *130*.

Hauser, M. (2011). A historic account of the invasion of Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Manag Sci, 67*(11), 1352-1357.

Hayes, K. R., & Barry, S. C. (2008). Are there any consistent predictors of invasion success? *Biological Invasions, 10*(4), 483-506.

Henry, G. A., & Stinchcombe, J. R. (2023). G-matrix stability in clinally diverging populations of an annual weed. *Evolution*, *77*(1), 49-62.

Hervé, M. (2016). RVAideMemoire: diverse basic statistical and graphical functions. R package v 0.9-55.

Hoffmann, A. A., & Parsons, P. A. (1988). The analysis of quantitative variation in natural populations with isofemale strains. *Génétique sélection évolution*, *20*(1), 87-98.

Hoffmann, A. A., & Shirriffs, J. (2002). Geographic variation for wing shape in Drosophila serrata. *Evolution*, *56*(5), 1068-1073.

Houle, D., & Meyer, K. (2015). Estimating sampling error of evolutionary statistics based on genetic covariance matrices using maximum likelihood. *Journal of Evolutionary Biology*, *28*(8), 1542-1549.

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D., & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, *287*(5451), 308-309.

Jolliffe, I. T. (2002). Graphical representation of data using principal components. In *Principal component analysis* (pp. 64-91). New York, NY: Springer New York.

Jones, A. G., Arnold, S. J., & Bürger, R. (2003). Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution*, *57*(8), 1747-1760.

Jones, A. G., Arnold, S. J., & Burger, R. (2004). Evolution and stability of the G-matrix on a landscape with a moving optimum. *Evolution*, *58*(8), 1639-1654.

Jones, A. G., Bürger, R., & Arnold, S. J. (2014). Epistasis and natural selection shape the mutational architecture of complex traits. *Nature communications, 5*(1), 1-10.

Karhunen, M., Merilä, J., Leinonen, T., Cano, J. M., & Ovaskainen, O. (2013). driftsel: an R package for detecting signals of natural selection in quantitative traits. *Molecular Ecology Resources*, *13*(4), 746-754.

Keller, S. R., & Taylor, D. R. (2008). History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecology letters*, *11*(8), 852-866.

Kilkenny, F. F., & Galloway, L. F. (2013). Adaptive divergence at the margin of an invaded range. *Evolution, 67*(3), 722-731.

Klingenberg, C. P., & Leamy, L. J. (2001). Quantitative genetics of geometric shape in the mouse mandible. *Evolution*, *55*(11), 2342-2352.

Klingenberg, C. P. (2003). Quantitative genetics of geometric shape: heritability and the pitfalls of the univariate approach. *Evolution*, *57*(1), 191-195.

Klingenberg, C. P. (2010). Evolution and development of shape: integrating quantitative approaches. *Nature Reviews Genetics*, *11*(9), 623-635.

Koch, J. B., Dupuis, J. R., Jardeleza, M.-K., Ouedraogo, N., Geib, S. M., Follett, P. A., & Price, D. K. (2020). Population genomic and phenotype diversity of invasive Drosophila suzukii in Hawai ‘i. *Biological Invasions*, 1-18.

Kruuk, L. E. (2004). Estimating genetic parameters in natural populations using the ‘animal model’. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1446), 873-890.

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution*, 314-334.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution*, 402-416.

Le Roy, C., Debat, V., & Llaurens, V. (2019). Adaptive evolution of butterfly wing shape: from morphology to behaviour. *Biological Reviews*, *94*(4), 1261-1281.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology & Evolution, 17*(8), 386-391.

Leinonen, T., Cano, J. M., & Merila, J. (2011). Genetics of body shape and armour variation in threespine sticklebacks. *Journal of Evolutionary Biology, 24*(1), 206-218.

Leinonen, T., McCairns, R. S., O'hara, R. B., & Merilä, J. (2013). QST–FST comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics, 14*(3), 179.

Lynch, M., & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*: Sinauer.

Martínez-Abadías, N., Mitteroecker, P., Parsons, T. E., Esparza, M., Sjøvold, T., Rolian, C., . Hallgrímsson, B. (2012). The developmental basis of quantitative craniofacial variation in humans and mice. *Evolutionary Biology*, *39*(4), 554-567.

Matsumura, S. (1931). *6000 illustrated insects of Japan-Empire*.

McGlothlin, J. W., Kobiela, M. E., Wright, H. V., Mahler, D. L., Kolbe, J. J., Losos, J. B., & Brodie, E. D., 3rd. (2018). Adaptive radiation along a deeply conserved genetic line of least resistance in Anolis lizards. *Evolution Letters, 2*(4), 310-322.

McGuigan, K. J. M. (2006). Studying phenotypic evolution using multivariate quantitative genetics. *Molecular Ecology, 15*(4), 883-896.

Merila, J., Bjorklund, M., & Gustafsson, L. (1994). Evolution of Morphological Differences with

Moderate Genetic Correlations among Traits as Exemplified by 2 Flycatcher Species (Ficedula, Muscicapidae). *Biological Journal of the Linnean Society, 52*(1), 19-30.

Merilä, J., & Crnokrak, P. (2001). Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology, 14*(6), 892-903.

Mezey, J. G., & Houle, D. (2003). Comparing G matrices: Are common principal components informative? *Genetics, 165*(1), 411-425.

Mezey, J. G., & Houle, D. (2005). The dimensionality of genetic variation for wing shape in Drosophila melanogaster. *Evolution*, *59*(5), 1027-1038.

Mitteroecker, P., & Bookstein, F. (2011). Linear Discrimination, Ordination, and the Visualization of Selection Gradients in Modern Morphometrics. *Evolutionary Biology, 38*(1), 100-114.

Monteiro, L. R., Duarte, L. C., & dos Reis, S. F. (2003). Environmental correlates of geographical variation in skull and mandible shape of the punar rat Thrichomys apereoides (Rodentia: Echimyidae). *Journal of Zoology, 261*(1), 47-57.

Morrissey, M. B., Hangartner, S., & Monro, K. (2019). A note on simulating null distributions for G matrix comparisons. *Evolution*, *73*(12), 2512-2517.

Myers, E. M., Janzen, F. J., Adams, D. C., & Tucker, J. K. (2006). Quantitative genetics of plastron shape in slider turtles (Trachemys scripta). *Evolution*, *60*(3), 563-572.

Olazcuaga, L., Loiseau, A., Parrinello, H., Paris, M., Fraimout, A., Guedot, C., ... & Gautier, M. (2020). A whole-genome scan for association with invasion success in the fruit fly Drosophila suzukii using contrasts of allele frequencies corrected for population structure. *Molecular biology and evolution*, 37(8), 2369-2385.

Ovaskainen, O., Karhunen, M., Zheng, C., Arias, J. M. C., & Merilä, J. (2011). A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics*, *189*(2), 621-632.

Pelabon, C., Hansen, T. F., Carter, A. J., & Houle, D. (2010). Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection. *Evolution*, *64*(7), 1912-1925.

Perrings, C., Dehnen-Schmutz, K., Touza, J., & Williamson, M. (2005). How to manage biological invasions under globalization. *Trends in Ecology & Evolution, 20*(5), 212-215.

Phillips, P. C., Whitlock, M. C., & Fowler, K. (2001). Inbreeding changes the shape of the genetic covariance matrix in Drosophila melanogaster. *Genetics*, *158*(3), 1137-1145.

Pitchers, W., Pool, J. E., & Dworkin, I. (2013). Altitudinal clinal variation in wing size and shape in African Drosophila melanogaster: one cline or many?. *Evolution*, *67*(2), 438-452.

Polly, P. D. (2008). Developmental dynamics and G-matrices: Can morphometric spaces be used to model phenotypic evolution?. *Evolutionary biology*, vol. 35, no 2, p. 83-96.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science, 13*(6), 288-294.

Puentes, A., Granath, G., & Agren, J. (2016). Similarity in G matrix structure among natural populations of Arabidopsis lyrata. *Evolution, 70*(10), 2370-2386.

Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology & Evolution, 29*(4), 233-242.

Robinson, M. R., & Beckerman, A. P. (2013). Quantifying multivariate plasticity: genetic variation in resource acquisition drives plasticity in resource allocation to components of life history. *Ecology Letters, 16*(3), 281-290.

Roff, D. (2000). The evolution of the G matrix: selection or drift? *Heredity*, *84*(2), 135-142.

Rogers, A. R., & Harpending, H. C. (1983). Population structure and quantitative characters. *Genetics*, *105*(4), 985.

Schafer, M. A., Berger, D., Rohner, P. T., Kjaersgaard, A., Bauerfeind, S. S., Guillaume, F., Fox, C. W., & Blanckenhorn, W. U. (2018). Geographic clines in wing morphology relate to colonization history in New World but not Old World populations of yellow dung flies. *Evolution*.

Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution*, *50*(5), 1766-1774.

Shaw, F. H., Shaw, R. G., Wilkinson, G. S., & Turelli, M. (1995). Changes in genetic variances and covariances: G whiz!. *Evolution*, 1260-1267.

Sikkink, K. L., Reynolds, R. M., Cresko, W. A., & Phillips, P. C. (2015). Environmentally induced changes in correlated responses to selection reveal variable pleiotropy across a complex genetic network. *Evolution, 69*(5), 1128-1142.

Siren, J., Ovaskainen, O., & Merila, J. (2017). Structure and stability of genetic variance-covariance matrices: A Bayesian sparse factor analysis of transcriptional variation in the three-spined stickleback. *Molecular Ecology, 26*(19), 5099-5113.

Steppan, S. J., Phillips, P. C., & Houle, D. (2002). Comparative quantitative genetics: evolution of the G matrix. *Trends in ecology & evolution*, *17*(7), 320-327.

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., ... & Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature ecology & evolution*, *1*(6), 0158.

Svensson, E. I., Arnold, S. J., Bürger, R., Csilléry, K., Draghi, J., Henshaw, J. M., Jones, A. G., De Lisle, S., Marques, D. A., & McGuigan, K. (2021). Correlational selection in the age of genomics. *Nature Ecology and Evolution*, 1-12.

Szűcs, M., Vahsen, M. L., Melbourne, B. A., Hoover, C., Weiss-Lehman, C., & Hufbauer, R. A. (2017). Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *Proceedings of the National Academy of Sciences*, *114*(51), 13501-13506.

Teplitsky, C., Robinson, M. R., & Merilä, J. (2014). Evolutionary potential. *Quantitative genetics in the wild*, 190.

Uesugi, A., Connallon, T., Kessler, A., & Monro, K. (2017). Relaxation of herbivore-mediated selection drives the evolution of genetic covariances between plant competitive and defense traits. *Evolution, 71*(6), 1700-1709.

Walsh, B., & Blows, M. W. (2009). Abundant Genetic Variation plus Strong Selection = Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annual Review of Ecology Evolution and Systematics, 40*, 41-59.

Walsh, B., & Lynch, M. (2018). *Evolution and selection of quantitative traits*. Oxford University Press.

Walter, G. M., Aguirre, J. D., Blows, M. W., & Ortiz-Barrientos, D. (2018). Evolution of genetic variance during adaptive radiation. *The American Naturalist*, *191*(4), E108-E128.

Walter, G. M. (2023). Experimental evidence that phenotypic evolution but not plasticity occurs along genetic lines of least resistance in homogeneous environments. *The American Naturalist*, *201*(4), E70-E89.

Wares, J. (2005). Mechanisms that drive evolutionary change. Insights from species introduction and invasions. *Species invasions: insights into ecology, evolution, and biogeography*, 229-257.

Whitlock, M. C. (2008). Evolutionary inference from QST. *Molecular ecology*, *17*(8), 1885-1896.

Wood, C. W., & Brodie, E. D., 3rd. (2015). Environmental effects on the structure of the G-matrix. *Evolution, 69*(11), 2927-2940.

FIGURES AND TABLES

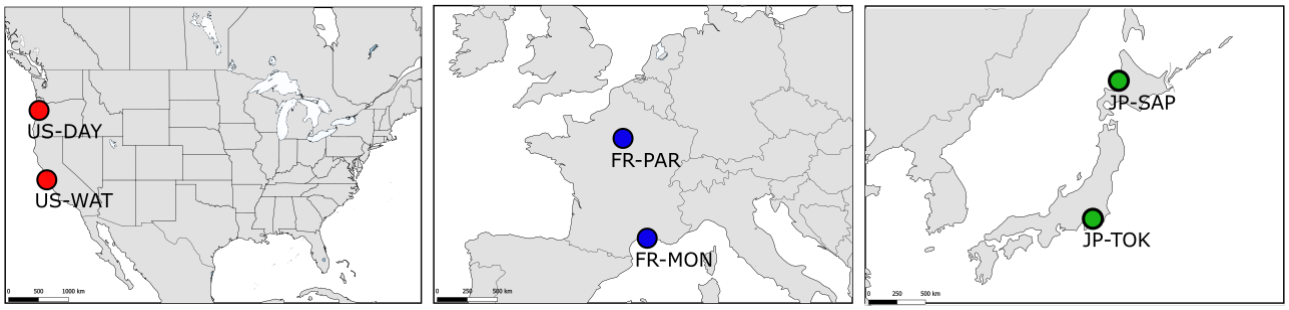

Figure 1. Sampling locations of the six *D. suzukii* populations. Adult *D. suzukii* were sampled from the wild in 3 countries (dark grey shading) from the native range in Japan (green circles) and the invasive range in France (blue circles) and the USA (red circles).

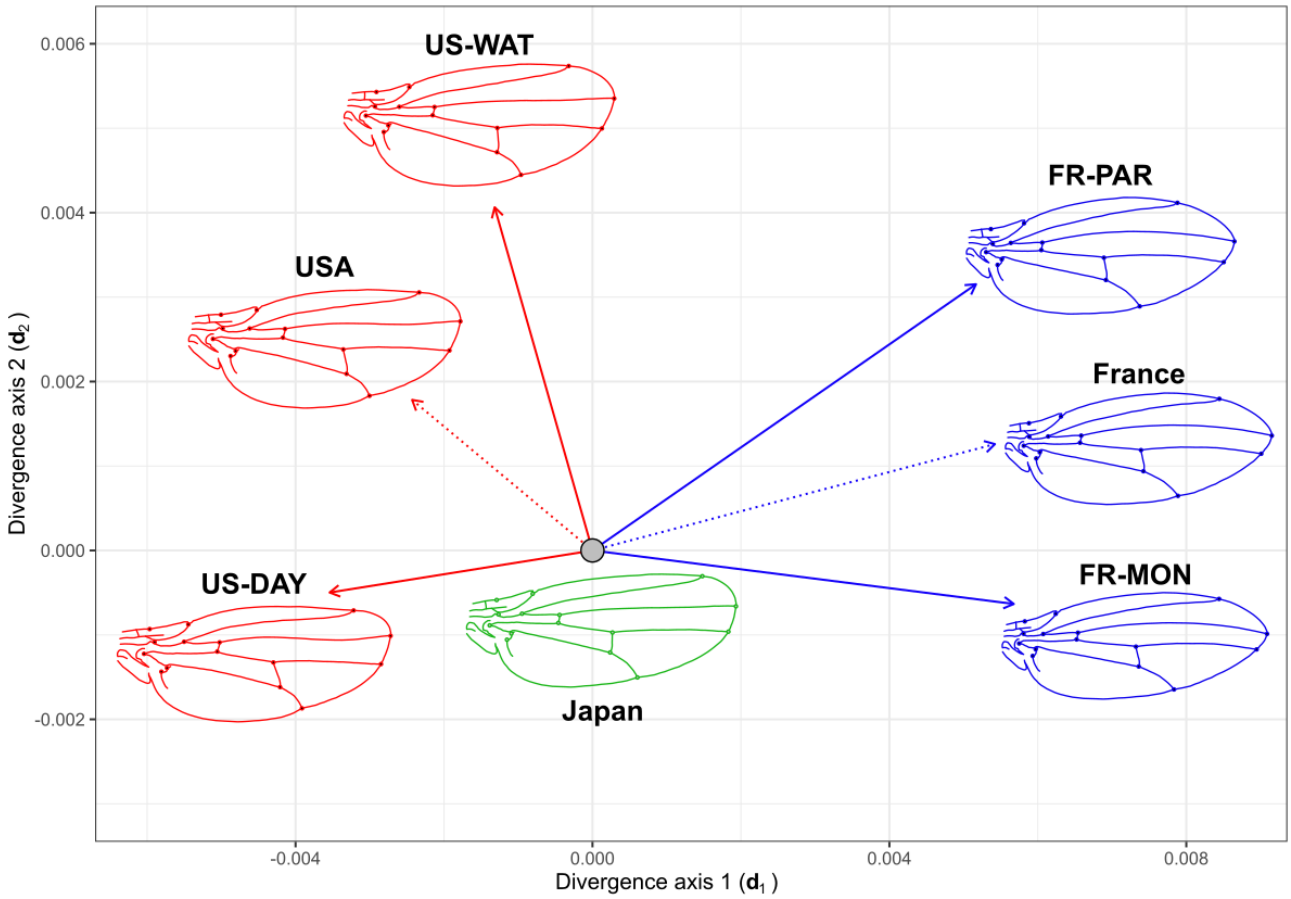

Figure 2. Phenotypic divergence of the invasive populations from their common ancestor. Graphical representation of the vectors of phenotypic divergence of each invasive population (solid line) from their hypothetical common ancestor (Japan, grey filled circle). The vectors are projected on the multivariate divergence space where d1 and d2 represent the first and second main axes of the multivariate divergent covariance matrix. The divergence vectors of the corresponding country matrices (dashed lines) are also shown. Colors correspond to Japanese (green), French (blue) and American (red) matrices. Wing shape outlines represent the mean shape of each group and show the wing shape evolution from the Japanese ancestor. Outlines were obtained via a thin plate spine warping of a reference outline using MorphoJ (Klingenberg, 2011), based on the position of landmarks and are used for visualization purposes only.

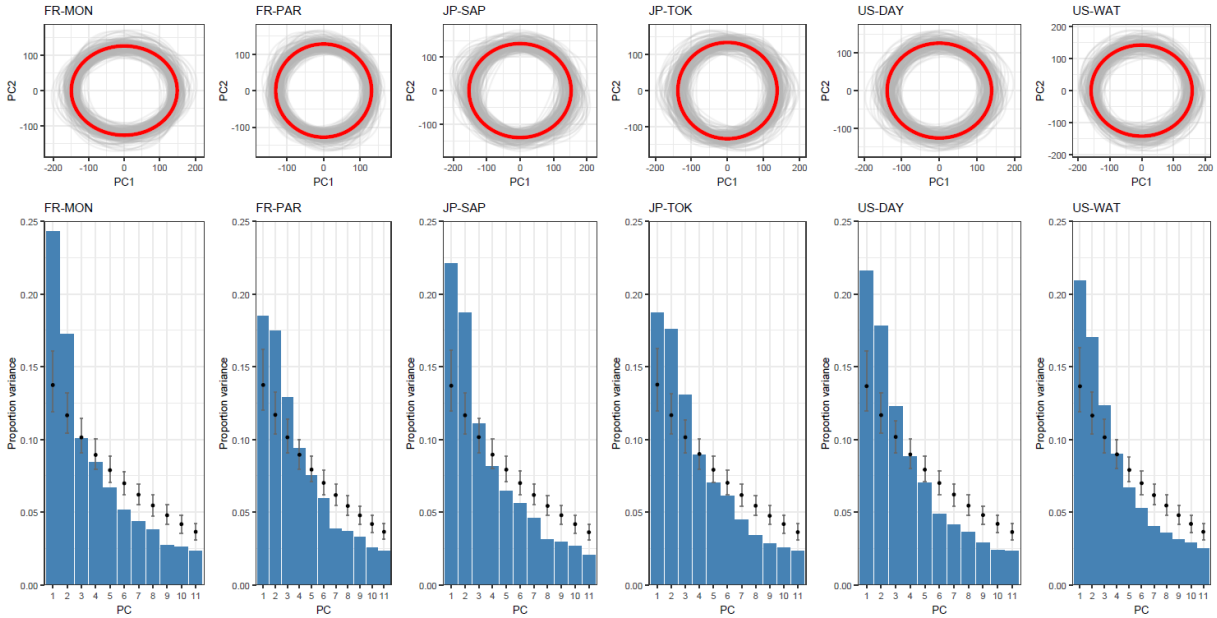

Figure 3. Graphical representation of the population-specific G-matrices. For each population-specific **G** matrix, the projection of the mean **G** matrix (red circles) onto the first two axes of the eigen decomposition (PC1 & PC2) are shown (top 6 panels). For each population, a sample of 200 ellipses corresponding to 200 random posteriors drawn from the MCMC outputs (grey ellipses) illustrate the uncertainty around our estimated **G** matrices. Results from the eigen decomposition of each matrix is shown by plotting the percentage of variance explained by the first 13 PCs (blue histograms; mean total variance explained across PC and populations = 91.22%) for both observed matrices, and null matrices (black filled points).

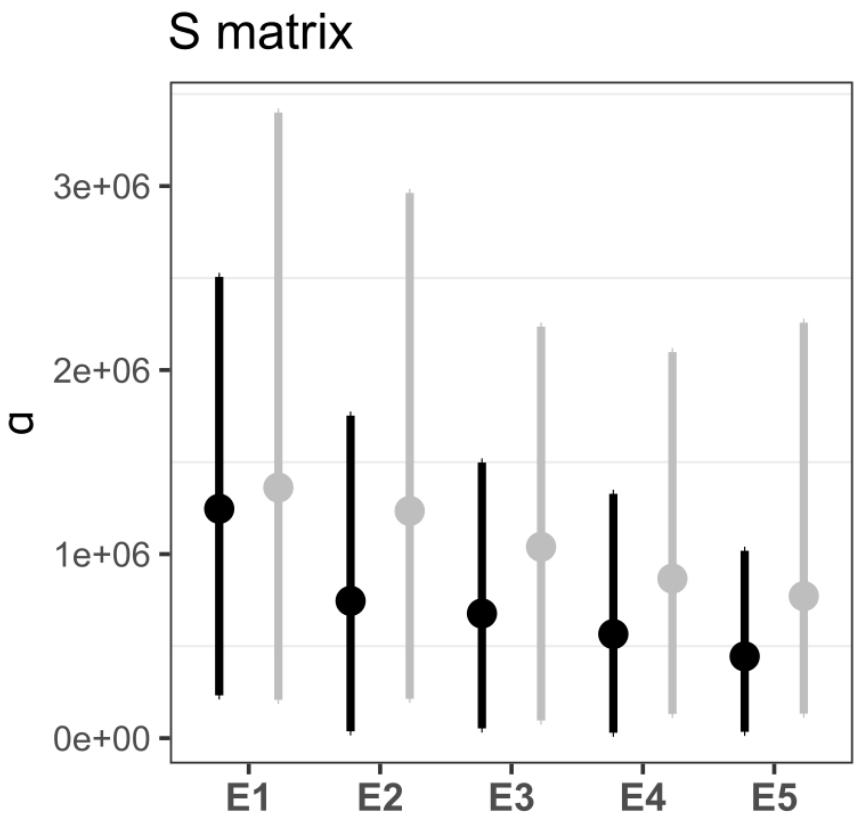

Figure 4. Results of the genetic covariance tensor analysis. Eigenvalues of the 5 observed eigentensors of **S** (black) are shown against the random expectation (grey). Overlapping of the 95% confidence intervals indicates that the observed **G** matrices are not more different than a random set of **G** matrices.

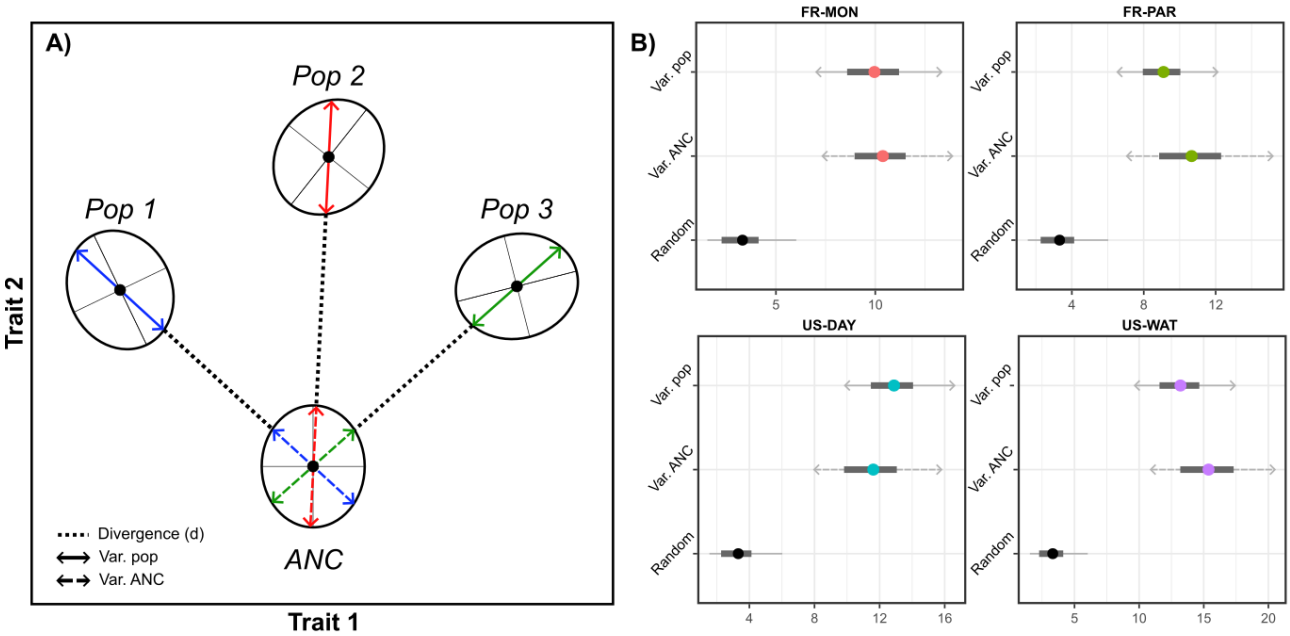

Figure 5. Percentage of genetic variance along divergence vectors. A) Conceptual figure for our test of selection based on the percentage of genetic variance along divergence vectors. In a bivariate trait space, population means (black filled circle) of three populations have divergence from a common ancestor (ANC). The within-population variance (Var.pop) corresponds to the amount of genetic variance in the direction of the phenotypic change between the ancestor (ANC) and the focal population. The ancestral variance (Var.ANC) corresponds to the amount of genetic variance in the ancestral **G** matrix, along that same divergence axis. B) For each population, the amount of genetic variation in the direction of divergence was computed (Var. pop.) and compared to the amount of variation in the ancestral G matrix in that direction (Var. ANC). The percentage of genetic variance along this axis is plotted (filled grey dot) with the 95% highest posterior density interval (black horizontal lines) next to the population code on the y axis. We hypothesize that selection would result in a reduction in genetic variance in the directions of divergence. This is clearly not observed, as none of the directions shows depleted genetic variance.

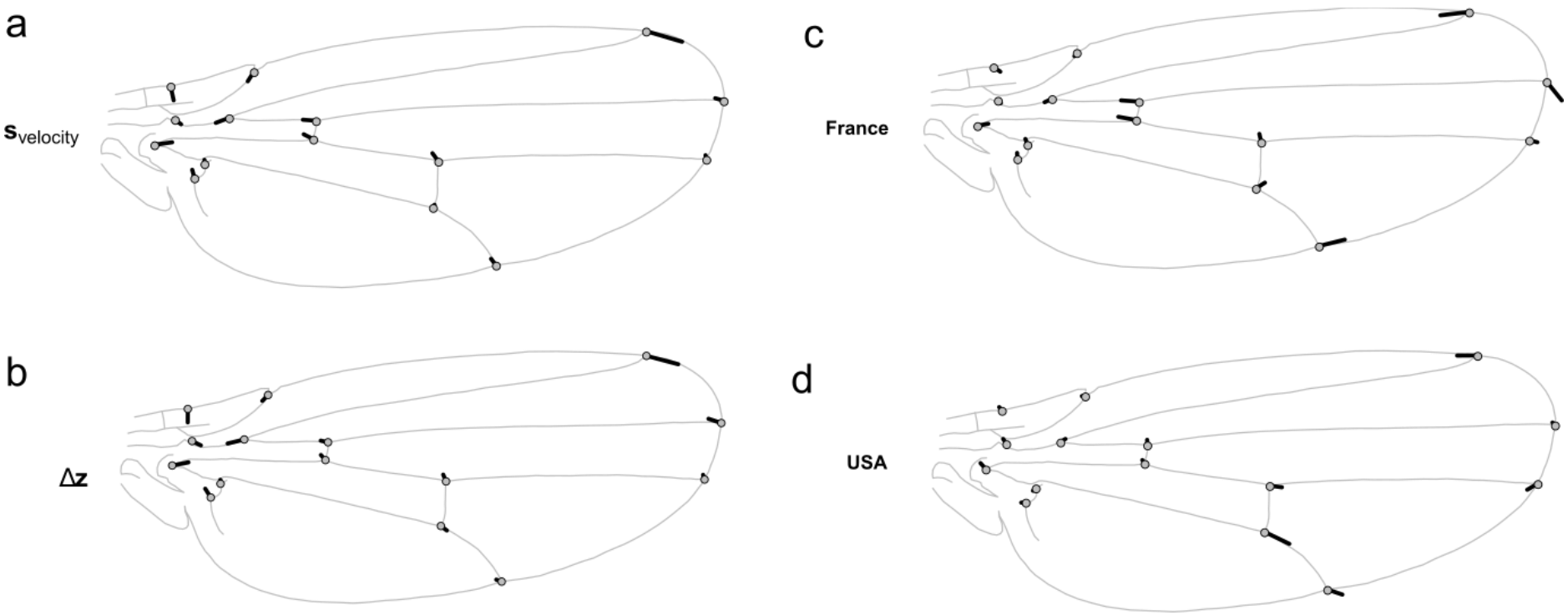

Figure 6. Response to a hypothetical selection compared to observed shape changes. Wing shape changes are displayed as vectors (black lines). a) selection differential, as defined by the shape difference between slow and fast fliers from Fraimout et al (2018). b) Average response to selection ($S_{velocity}$) based on the MCMC estimates of the ancestral **G** and **P** matrices. c) Average phenotypic divergence of the French populations from the ancestor. d) Average phenotypic divergence of the American populations from the ancestor.

Population	JP-TOK	JP-SAP	US-DAY	US-WAT	FR-PAR
JP-SAP	1.03 [0.83:1.3]				
US-DAY	0.88 [0.69:1.08]	0.87 [0.66:1.03]			
US-WAT	1.17 [0.95:1.45]	1.14 [0.9:1.38]	**1.32 [1.09:1.65]**		
FR-PAR	0.93 [0.73:1.13]	0.89 [0.7:1.07]	1.05 [0.83:1.29]]	**0.79 [0.63:0.94]**	
FR-MON	0.93 [0.73:1.11]	0.88 [0.7:1.06]	1.04 [0.83:1.26]	**0.76 [0.62:0.92]**	0.95 [0.79:1.2]

Table 1. Pairwise comparison of G matrices' volumes. For each pair of **G** matrix the posterior mode of the ratio between volumes is shown along with the Highest Posterior Density (HPD) interval. Bold values correspond to significant differences between two matrices volumes whether the ratio is significantly > 1 (row matrix is larger) or < 1 (row matrix is smaller).