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Phenotypic plasticity of *Drosophila suzukii* wing to developmental temperature: implications for flight

Antoine Fraimout[1,*], Pauline Jacquemart[1], Bruno Villarroel[1,2], David J. Aponte[1,3], Thierry Decamps[2], Anthony Herrel[2], Raphaël Cornette[1] and Vincent Debat[1]

ABSTRACT

Phenotypic plasticity has been proposed as a mechanism that facilitates the success of biological invasions. In order to test the hypothesis of an adaptive role for plasticity in invasions, particular attention should be paid to the relationship between the focal plastic trait, the environmental stimulus and the functional importance of the trait. The *Drosophila* wing is particularly amenable to experimental studies of phenotypic plasticity. Wing morphology is known for its plastic variation under different experimental temperatures, but this plasticity has rarely been investigated in a functional context of flight. Here, we investigate the effect of temperature on wing morphology and flight in the invasive pest species *Drosophila suzukii*. Although the rapid invasion of both Europe and North America was most likely facilitated by human activities, *D. suzukii* is also expected to disperse actively. By quantifying wing morphology and individual flight trajectories of flies raised under different temperatures, we tested whether (1) invasive populations of *D. suzukii* show higher phenotypic plasticity than their native counterparts, and (2) wing plasticity affects flight parameters. Developmental temperature was found to affect both wing morphology and flight parameters (in particular speed and acceleration), leaving open the possibility of an adaptive value for wing plasticity. Our results show no difference in phenotypic plasticity between invasive and native populations, rejecting a role for wing plasticity in the invasion success.

KEY WORDS: ***Drosophila suzukii*, Phenotypic plasticity, Flight, Wing aspect ratio, Geometric morphometrics, Wing shape**

INTRODUCTION

Phenotypic plasticity – the capacity of a genotype to produce different phenotypes in different environments – is a ubiquitous property of organisms (West-Eberhard, 1989, 2003). Its evolutionary role has been recently reassessed and it is now considered an important component of adaptive evolution to environmental change (e.g. Pigliucci, 2005; Merilä and Hendry, 2014; Schneider and Meyer, 2017). In particular, adaptive plasticity has been suggested to facilitate biological invasions (e.g. Geng et al., 2007; Lande, 2015), by allowing phenotypic adjustment to new conditions after introduction when genetic variation has been

depleted by demographic bottlenecks or genetic drift (Geng et al., 2007, 2016). If plasticity indeed plays an important role in invasions, one would thus expect invasive species to be more plastic than non-invasive ones (Richards et al., 2006; Davidson et al., 2011) and, similarly, within species, invasive populations to be more plastic in their new range than in their native ones (Parker et al., 2013; Foucaud et al., 2016). Experimentally, these predictions can be tested by measuring the plastic variation of single or multiple traits in response to a controlled environmental stimulus and comparing reaction norms of native and invasive populations (Lee et al., 2007). However, plasticity may produce phenotypic variation in non-adaptive directions (e.g. Ghalambor et al., 2007). Linking phenotypic plasticity to invasion success therefore requires the focal trait to be physiologically or ecologically advantageous for the invasive individuals (Molina-Montenegro et al., 2012) and the environmental cue to reflect ecological significance for the species (Forsman, 2015).

Among the factors which can trigger plastic responses and be experimentally manipulated, temperature has been shown to affect both morphological and life-history traits under laboratory conditions in a variety of biological models (e.g. Rhen and Lang, 1995; David et al., 1997; Atkin et al., 2006; Smith et al., 2013). For ectotherm organisms such as insects, temperature is a relevant ecological parameter as it has a strong influence on the life-cycle and geographic distribution in the wild (e.g. Deutsch et al., 2008; Régnière et al., 2012). In *Drosophila*, extensive laboratory work has shown that developmental temperature – the temperature experienced by the individual from egg to adult – has strong effects on phenotypes, including both morphology (e.g. negative correlation between temperature and thorax length, pigmentation; e.g. Barker and Krebs, 1995; Gibert et al., 2000; Shingleton et al., 2009) and life-history traits (e.g. lower fecundity and increased longevity in *D. melanogaster* at lower temperature; e.g. Nunney and Cheung, 1997). Wing morphology in particular is well known to be thermally plastic (e.g. David et al., 1994, 2005; Partridge et al., 1994; Imasheva et al., 2000; Debat et al., 2003, 2009; Gilchrist et al., 2004; Bubliy and Loeschcke, 2005; Pitchers et al., 2013; Torquato et al., 2014; Przybylska et al., 2016). *Drosophila* wing shape has been shown to be a phylogenetically conserved trait but also highly evolvable in the laboratory (Weber, 1992; Houle et al., 2003), suggesting strong stabilizing selection on this trait (Hansen and Houle, 2004, 2008). However, geographic variation for wing morphology in wild *Drosophila* populations also suggests adaptive evolution to latitudinal and altitudinal (thus environmental) clines (e.g. Bitner-Mathé and Klaczko, 1999; Huey et al., 2000; Gilchrist et al., 2004; Gilchrist and Huey, 2004; Pitchers et al., 2013). Whether wing size and shape plasticity is adaptive or not is a contentious issue. Clinal divergence for thermal plasticity in wing morphology has been documented and these plastic clines are in the same direction as the genetic clines, suggesting an adaptive value

[1]Institut de Systématique, Evolution, Biodiversité, ISYEB–UMR 7205–CNRS, MNHN, UPMC, EPHE, Muséum National d'Histoire Naturelle, Sorbonne Universités, 57 rue Cuvier, CP 50, 75005 Paris, France. [2]Mécanismes Adaptatifs et Evolution, MECADEV–UMR 7179, CNRS, MNHN, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France. [3]Department of Cell Biology & Anatomy, University of Calgary, Calgary AB, Canada.

*Author for correspondence (fraimout.antoine@gmail.com)

A.F., 0000-0003-4552-3553

for this thermal plasticity (e.g. Pétavy et al., 1997; Morin et al., 1999; Pitchers et al., 2013). Added to its role in locomotion by flight, the *Drosophila* wing thus appears as a relevant focal trait for a specific test of the relationship between phenotypic plasticity, morphology and performance.

Flight in *Drosophila* has been studied under contrasting experimental contexts – with flight acquisitions performed at different temperatures (e.g. Marden et al., 1997; Card and Dickinson, 2008) – and is expected to be influenced by temperature through altered muscle contraction kinetics (Lehmann, 1999; Dillon and Frazier, 2006; Frazier et al., 2008). Besides, several aspects of the wing size and shape have been proposed to affect flight performance under different thermal conditions. Regarding wing size, Gilchrist and Huey (2004) proposed that reduced wing-loading (i.e. the wing size to weight ratio) in *Drosophila subobscura* from cold climates could compensate for reduced efficiency of flight muscles in cold air and thus relate to adaptive plasticity in natural populations.

When considering the effect of wing shape on flight performance, several descriptors have been used in the literature. The simplest is the wing aspect ratio (AR), which estimates wing elongation relative to area ($AR=4R^2/S$; where R=total wing length and S=wing area; Dudley, 2002). This crude descriptor of wing shape has been found to correlate with flight performance (Dudley, 2002) and has been described in several *Drosophila* species (e.g. Bubliy and Loeschcke, 2005). Azevedo et al. (1998) hypothesized in *D. melanogaster* that a higher wing AR could be advantageous for flight at low temperatures (via a compensatory effect of lower wing-beat frequencies in the cold), a result that was later on interpreted by Frazier et al. (2008) as adaptive developmental plasticity. AR, however, has been shown to be weakly associated with aerodynamic forces (e.g. Usherwood and Ellington, 2002; Luo and Sun, 2005; Muijres et al., 2017) and aerodynamically more relevant descriptors of wing shape have been proposed (Weis-Fogh, 1973; Ellington, 1984). The second moment of area (S2), which estimates the distribution of wing area along the wing axis, in particular, directly scales with the flight force produced during flapping (e.g. Muijres et al., 2017). Wings with a high S2 (i.e. enlarged wing tips) will produce higher aerodynamic forces. However, results from these studies raise questions on how exactly are wing morphology and flight associated. Specifically, whether flight is affected by more subtle wing shape variation and which specific flight parameters are influenced by differences in wing morphology is unknown.

Here, we investigate the effect of developmental temperature on the wing morphology of the invasive pest species *Drosophila suzukii* and test whether changes in wing size and shape affect flight parameters. The spotted-wing *D. suzukii* is an Asian fruit fly species from the *melanogaster* group (Kopp and True, 2002). The species is a particularly successful invader that has colonized more than 20 countries across Europe, and North and South-America (Hauser, 2011; Calabria et al., 2012; Deprá et al., 2014), from its native Asian range in less than a decade (see Asplen et al., 2015 for a detailed review). Although rapid trans-continental migration is most probably human mediated (i.e. global trade of fruits, to which *D. suzukii* is associated; Lee et al., 2011), genetic data and release/recapture experiments of marked individuals suggest that *D. suzukii* actively disperses through flight (Tait et al., 2018; Fraimout et al., 2017).

Using geometric morphometrics and semi-automated tracking of flights obtained from high-speed video cameras, we respectively analyze wing size and fine-scale shape, and flight trajectories of

D. suzukii individuals reared at different temperatures. We assess flight performance as the set of flight parameters derived from these trajectories (see Materials and Methods). We test the hypothesis that temperature-induced morphological variation in the wing affects flight parameters. Furthermore, by contrasting *D. suzukii* populations derived from the native and invasive ranges, we test whether invasive populations exhibit higher phenotypic plasticity for wing morphology than native populations.

MATERIALS AND METHODS
Study populations and rearing
Drosophila suzukii (Matsumura 1931) adults were sampled during summer 2014, using banana bait traps and net swiping at three localities representative of their global distribution range in Japan (native range), the USA and France (invasive range). For each country, a northern population was sampled to allow comparisons between thermally comparable conditions. The Japanese population was sampled in Sapporo (Hokkaido), the US population was sampled in Dayton (Oregon) and the French population was sampled in Paris. Fly stocks were established by ten isofemale lines per locality by performing single matings in separated rearing vials and expanding F1 offspring in a subsequent series of replicate vials (Hoffmann and Parsons, 1988; David et al., 2005; Madi-Ravazzi et al., 2017). Stock populations were kept at 22°C on a rearing medium consisting of corn starch and yeast with antibiotics (hydroxy-4 benzoate) added. Prior to the experiment, flies from each population were transferred to oviposit in two batches of 20 new vials (i.e. 10 vials per batch) for 24 h. For each population, after oviposition was ensured (i.e. presence of eggs in the medium), parent flies were removed and the two batches were separately placed in two incubators at 16 and 28°C and one batch kept at stock temperature (22°C). Thus, each population was represented by 10 isofemale lines reared at three experimental temperatures, resulting in 30 lines per geographic population. Lines were randomly positioned in incubators and kept at the experimental temperatures until 2 days after emergence to ensure sexing and optimal flight ability (Dillon and Frazier, 2006). For each line from each population, one male *D. suzukii* was then randomly chosen for flight analysis, resulting in a total of 89 individual flies that were filmed and measured. Flight acquisition procedures started ca. 4 months after the first sampling date, resulting in different durations in the lab for Japanese, French and North-American flies (respectively four, three and two generations). We assumed this small number of generations in lab conditions insufficient to affect the flight abilities especially because only flies capable of flying were chosen. Thus, if flies were to be impaired in their flight capacity by the time spent in the lab, they would not have been included in the present study.

Flight acquisition and analysis
Flies were released at one side of a circular and transparent Plexiglas chamber covered on one side by white paper to maximize contrast and facilitate subsequent digitization (Fig. S1). Each fly was released individually and allowed to fly freely in the chamber. Room temperature was not controlled but measured at 21°C. Unresponsive flies were stimulated by touching them briefly with a fine paint brush. We considered a successful flight to be at least 3 s without interruption, and three successful flights were recorded for each individual. Flight duration varied among individuals and replicate flights (min: 3.92 s, max: 57 s, mean=20.16 s). Flies were then killed and preserved in 70% ethanol. Flights were recorded using three synchronized high-speed cameras (Prosilica GE680, Allied Vision

Technologies GmbH, Stadtroda, Germany) set at 200 frames s⁻¹. Two cameras were set up in dorsal view and one camera was set up in oblique frontal view. Thus, the fly was visible in at least two of the three views during the whole recorded sequence. Cameras were calibrated and scaled using a direct linear transformation (DLT) routine (Hartley and Sturm, 1995) based on the digitization of a moving black-and-white checkerboard (i.e. multiple positions acquired) composed of ten 1×1 cm squares. The position of the fly was digitized and the screen coordinates were calculated on the three synchronized views using a custom MATLAB routine (Loco 2.8). Next, flight paths were smoothed with a low-pass Butterworth filter (Fig. S2) and the following flight parameters were calculated using a custom-written R script: sinuosity (defined as the ratio of the distance between the initial and final positions over the actual flight path length), peak flight speed, acceleration and deceleration, smallest and biggest angular turns and their associated peak angular speed, acceleration, and deceleration. These flight parameters were used to define the flight performance of each individual. The highest values out of the three flights were retained for each parameter with the exception of the smallest turn angle for which we retained the minimal value. The analysis of selective pressures acting on morphology is a difficult task, in particular because of the complex link between morphology, behaviour and performance. In this context, it has been proposed that maximal performance should be particularly informative (Losos et al., 2002). One can for instance think of track runners slightly differing in height who might walk at the same pace when unchallenged but perform differently when pushed to their maximal running speed. Accordingly, we reasoned that subtle morphological differences in wing morphology would be more likely to have an effect on extreme values of flight parameters rather than on their means, and we thus primarily focused on such extreme values. However, as our experimental setup was not particularly challenging for flies, it is unclear how such extreme values actually reflect maximum performance. Therefore, we also ran the analyses on their mean values. Finally, because flight duration strongly varied across flights and individuals, we tested for an association between flight parameters and flight duration using uni- and multivariate regressions. To ensure comparability, absolute values of the flight parameters were log-transformed prior to analysis.

Measurements of wing size, shape and aerodynamic parameters

Wing shape data were obtained using landmark-based geometric morphometrics. Wings were mounted on 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 then acquired with a Leica DFC 420 digital camera mounted on a Leica Z6 APO microscope. Fifteen landmarks were defined on the dorsal face of the right wing (see Fig. 1) and digitized using a custom plugin implemented in ImageJ software (v.1.51.k; Schneider et al., 2012). Generalized Procrustes superimposition was used to extract shape information from the landmark coordinates (e.g. Rohlf and Slice, 1990; Dryden and Mardia, 1998). A principal component analysis (PCA) was first applied to the set of 30 coordinates and the non-null 26 components were conserved for the shape analyses. Centroid size was used as a size variable in the subsequent analyses. Other morphological parameters (wing length and wing area) were obtained using the wingImageProcessor program (http://www.unc.edu/~thedrick).

In order to get functional insights from the effects of variation in wing morphology on flight, we calculated three parameters classically used to characterize wing aerodynamic properties:

Fig. 1. Positions of the 15 landmarks used to quantify the wing shape of *Drosophila suzukii*.

the AR, the S2 and the wing:thorax size ratio. AR and the S2 were calculated following Ellington (1984) and using the wingImageProcessor program (http://www.unc.edu/~thedrick). As flies were stored in ethanol after recorded flights, estimation of fresh weight was not feasible in order to calculate wing loading. We thus used the wing:thorax ratio as a predictor of wing loading (Pétavy et al., 1997). The wing:thorax ratio is inversely related to wing loading (Azevedo et al., 1998), which is important for producing lift during flight (Azevedo et al., 1998; Gilchrist and Huey, 2004). We calculated the wing:thorax ratio as (wing area)/(thorax length). For each individual, thorax length was defined from the left side of the body as the length between the posterior tip of the scutellum to the most anterior edge of the thorax. We used a Nikon DS-Fi1 camera mounted on a Nikon SMZ800 microscope to acquire pictures and measurements were made using ImageJ software (v.1.51; Schneider et al., 2012). The repeatability of thorax length measurement (based on nine repeated measurements of 30 randomly chosen individuals) was 99%.

Statistical analyses

All statistical analyses were performed in R (http://www.R-project.org/). We first performed PCA and linear discriminant analyses (LDA) on both shape and flight datasets to visually investigate variation, using temperature and population as grouping factors in the LDA. We then tested the effect of developmental temperature and geographic origin on wing morphology and flight parameters. For wing morphology, we performed uni- and multivariate analyses of variance (ANOVA and MANOVA) on all measurements of wing size (i.e. centroid size), shape (i.e. non-null PC scores) and aerodynamic parameters described above with population and temperature as main effects. Differences in wing allometry within and between groups were investigated by performing a Procrustes ANOVA with a permutation procedure using the *procD.allometry* function implemented in the *geomorph* R-package (v.3.0.3; Adams et al., 2017). The effect of temperature and population on flight parameters was tested by performing a MANOVA on the combined flight parameters and subsequent ANOVAs for each variable independently. We then estimated the degree of covariation between flight parameters and wing shape data using the Escoufier's RV coefficient (Escoufier, 1973; Klingenberg, 2009) and a two-blocks partial least squares (PLS)
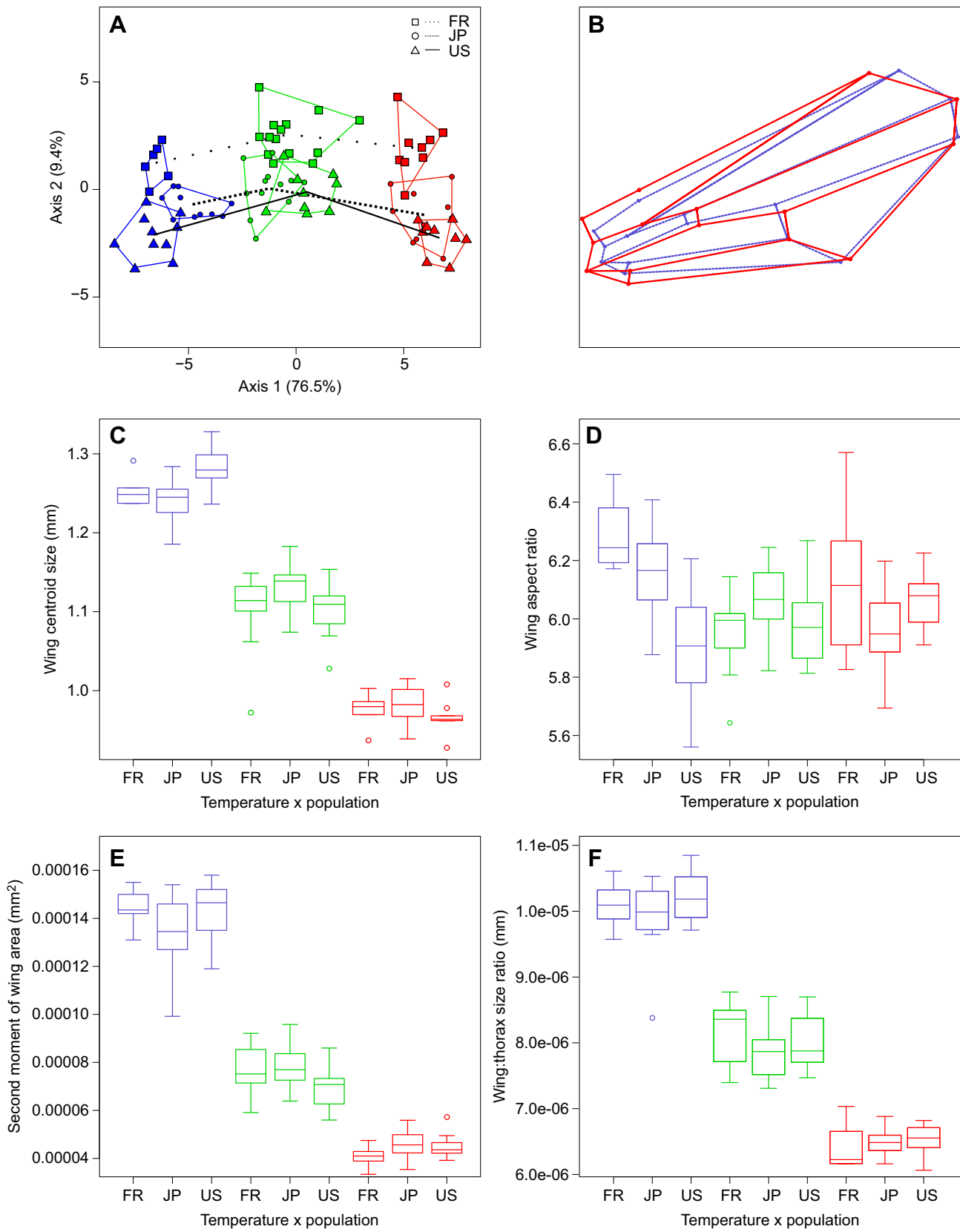
Fig. 2. See next page for legend.

**Fig. 2. Effect of developmental temperature on different aspects of
D. suzukii wing morphology.** The effect of developmental temperature on all
three populations (FR: Montpellier, France; JP: Sapporo, Japan: US: Dayton,
USA) on (A,B) wing shape as represented by (A) linear discriminant analysis
(LDA) and (B) the visualization of wing shape changes along the first LDA axis;
(C) wing centroid size; (D) wing aspect ratio; (E) the second moment of wing
area and (F) wing:thorax size ratio. Colours of the graphs represent the three
experimental temperatures: blue=16°C, green=22°C, red=28°C. Black lines
represent the shape reaction norms on the first discriminant plan. (B) The
visualization plots correspond to negative and positive values along the
discriminant axis. For the sake of simplicity, extreme wing shapes are coloured
according to the associated temperature (blue=16°C, red=28°C).

regression (Rohlf and Corti, 2000). To visualize the shape changes
associated with particular components of flight, multivariate
regressions of each of these parameters on shape data were
applied (e.g. Monteiro, 1999).

RESULTS
Mean and maximal values of flight parameters produced highly
similar results. Only those obtained with maximal values are
reported below.

Effect of temperature on wing morphology and aerodynamic
properties
As expected, temperature had a strong effect on wing morphology
(Fig. 2). A negative relationship between wing centroid size and
temperature was observed for all populations (Fig. 2C), with flies
raised at a cooler temperature having larger wings. The amplitude of
the plastic response in size was higher for the US population
compared with the Japanese population (i.e. an interaction between
temperature and population was detected in the ANOVA,
$F_{4,80}=3.822$; $P=0.007$; Fig. 2C). The two main factors
(population and temperature) each had an effect on wing shape
(Fig. 2A). The lack of interaction between temperature and
population indicates that no difference in plasticity for wing shape
between populations was detectable. Individuals from all three
populations were thus pooled together for further shape/flight
analyses. As wing shape can be influenced by size (i.e. allometry,
e.g. Debat et al., 2003; Bolstad et al., 2015) we performed a
MANOVA on the residuals from a multivariate regression of size on
shape (i.e. the non-allometric component of shape). This procedure
was valid as we did not detect any significant departure of the
within-group allometries from the common pattern of allometry
(Fig. S3; see Klingenberg, 2016 for a review). There was no effect of
temperature on shape residuals after extraction of the allometric
component (Table S1), indicating that the previously observed
effect of temperature on wing shape was mostly allometric. No
effect of temperature and population on the wing AR could be
detected (ANOVA, $F_{2,83}=1.7$; $P=0.189$; ANOVA, $F_{2,83}=2.466$;
$P=0.091$; Fig. 2D). Temperature had a strong effect on wing:thorax
ratio (ANOVA, $F_{2,76}=198.6$; $P<0.001$; Fig. 2F), the wing:thorax
ratio being negatively correlated with temperature. Consequently,
wing loading – which is inversely related to wing:thorax ratio –
increased with temperature. There was no difference in wing:thorax
ratio among populations or in the plastic response among
populations (ANOVA, $F_{4,70}=0.844$; $P=0.502$). Temperature had a
strong effect on S2 (ANOVA, $F_{2,85}=621.9$; $P<0.001$; Fig. 2E), the
latter being negatively correlated with temperature.

Effect of temperature on flight parameters
We first investigated the correlations across flight parameters.
Unsurprisingly, speed and acceleration/deceleration were strongly

correlated. The same was found for angular parameters, but the two
sets of parameters were only mildly correlated (not shown).
Sinuosity and the smallest angular change were not correlated
with any other parameter. We investigated the effects of temperature
and population on flight parameters. A standard MANOVA
indicated an interaction between both effects (Wilks' $\lambda=0.408$;
$P=0.012$), although the population effect was not significant
(Wilks' $\lambda=0.652$; $P=0.076$). However, when performing a
permutational MANOVA (McArdle and Anderson, 2001;
Anderson, 2001), the interaction between the two effects was lost
($F_{4,80}=0.518$; $P=0.804$). Considering this as an indication of a weak
interaction at best, we decided to pool all individuals from all three
populations to simplify further analyses. Although differentiation
was rather weak, temperature had a significant effect on
flight performance (MANOVA, Wilks' $\lambda=0.614$; $P=0.013$; and
permutational MANOVA, $F_{1,88}=5.348$; $P=0.003$). We then
investigated which individual flight variable was affected by
temperature. Temperature had a significant effect on velocity,
acceleration, deceleration and angular acceleration (Table S2). More
precisely, significant differences were observed for flies raised at
16°C compared with the two other experimental populations, the
flies reared in colder temperature being capable of higher
acceleration and faster flights (Fig. 3). Regression of flight
parameters on flight duration showed no effect of flight time on
velocity and acceleration ($F_{1,84}=3.395$, $r^2=0.027$, $P=0.068$;
$F_{1,84}=0.213$, $r^2=-0.009$, $P=0.645$, respectively). Lastly, we found
no association between temperature and flight duration (ANOVA,
$F_{2,83}=1.747$; $P=0.181$) as variation in flight duration was randomly
distributed across treatments.

Association between shape and flight
Running multivariate regressions of individual flight parameters on
shape data, we found that velocity and acceleration had the strongest
effect. The shape change associated with increased speed is shown
on Fig. 3. Flies reared at the cooler temperature (i.e. fastest flies, in
blue on Fig. 3) were characterized by narrower proximal sections of
the wing (i.e. distance between landmarks 1–3 and 5–7) and a
slightly broader wing tip (distal movement of landmarks 12 and 14;
Fig. 2B). Shape change was less pronounced for acceleration
(Fig. 3). The RV test revealed a significant covariation between
shape and flight (RV=0.084; $P=0.03$). The PLS analysis confirmed
this result, showing that the covariation between shape and flight is
driven by temperature (Fig. 4). The flight component of the PLS
analysis (PLS block 2) is clearly driven by speed and acceleration.
Unsurprisingly, the shape change associated with the first PLS axis
is extremely similar to that found for the association with velocity,
the fastest flies presenting the most contracted wing base and
broadened wing tip, and being found at the lowest temperature. To
account for the potential effect of differences in flight duration on
the shape/flight association, we also performed both tests using
residuals from a multivariate regression of maximal flight
parameters on flight time, and obtained highly similar results
(RV=0.089; $P=0.01$; PLS plot not shown).

DISCUSSION
We studied the effect of developmental temperature on *D. suzukii*
wing morphology and how this phenotypic plasticity may affect
specific flight parameters. As the focal trait is a crucial aspect of
phenotypic plasticity experiments (Forsman, 2015), we chose to
focus on wing morphology, a highly plastic trait in *Drosophila*,
involved in flight (David et al., 1997; Ray et al., 2016) and courtship
(e.g. Ewing, 1964; Yeh and True, 2014) behaviours. We thus

analyzed the effect of temperature on the individual association of high dimensional wing shape data and individual flight parameters extracted from 3D trajectories. The high quality of such individual data comes at the price of a relatively low sample size, limiting the statistical power of our analyses. One should thus consider the occasional lack of statistical effect cautiously (e.g. differences in plasticity among populations). This study nevertheless provides one of the most extensive datasets, allowing one to investigate the relationship between individual morphology and the corresponding flight parameters (see also Ray et al., 2016).

Our results show that, regardless of the population, developmental temperature affects flight parameters in *D. suzukii*, with flies reared at the coldest experimental temperature (i.e. 16°C) showing the highest flight velocity and acceleration. Developmental temperature also has a strong effect on both wing size and shape, as well as on S2 and wing:thorax ratio. In the light of these results, we propose several interpretations explaining how developmental temperature may affect flight parameters.

The question of whether the effect of temperature on flight is indeed mediated by wing plasticity or rather involves other plastic traits remains open. Particularly, we do not have any information on the effect of developmental temperature on neuromuscular morphology and activity, parameters that are crucial for insect flight (Sato et al., 2015; Lindsay et al., 2017). In our experiment we

could not disentangle the direct effects of developmental temperature from those mediated by wing size and shape plasticity, partly due to sample size and statistical power limitation. It is thus conceivable that the detected flight difference might result from sources other than differences in wing morphology. Nevertheless, we argue that our results suggest that wing plasticity might indeed impact flight performance. Joint effects of temperature and wing morphology on flight performance have previously been reported for *D. melanogaster* (Barnes and Laurie-Ahlberg, 1986; Dillon and Frazier, 2006; Frazier et al., 2008). Cold-reared flies are expected to show increased lift performance in cold air through higher aerodynamic forces yielded by elongated wings and lower wing loading (Ellington, 1984; Frazier et al., 2008). A first possibility is that plasticity of wing size is sufficient to explain differences in flight. Studying *Drosophila subobscura*, Gilchrist and Huey (2004) proposed that such plasticity has functional significance, the reduced wing loading in flies reared in cold conditions compensating for the effect of cold air on wing muscles. Using wing:thorax size ratio as a proxy for wing loading, we report, in agreement with these studies, that *D. suzukii* reared at the lowest temperature indeed show the lowest wing loading (i.e. highest wing:thorax ratio; Fig. 2F). A specificity of our study is that our flies – cold-, medium- and warm-reared – all flew at an intermediate temperature (21°C). It is
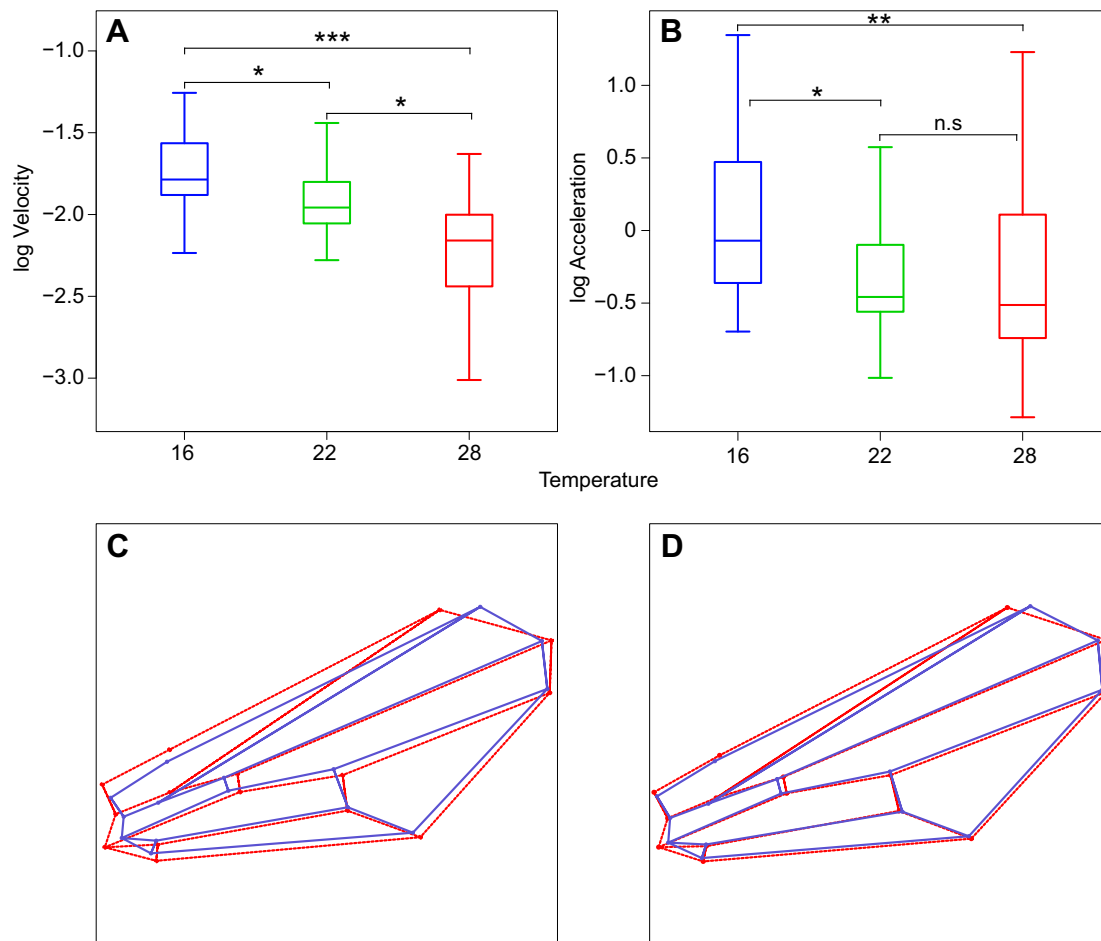

Fig. 3. Temperature effect on flight velocity and acceleration. Boxplots represent the differences in velocity (A) and acceleration (B) between the three developmental temperatures: blue=16°C, green=22°C, red=28°C. (C,D) The visualization plots correspond to negative and positive values along the first axis of regression and represent the wing shapes associated to velocity (C; m s^{-1}) and acceleration (D; m s^{-2}). For the sake of simplicity, extreme wing shapes are coloured according to the associated temperature (blue=16°C, red=28°C).
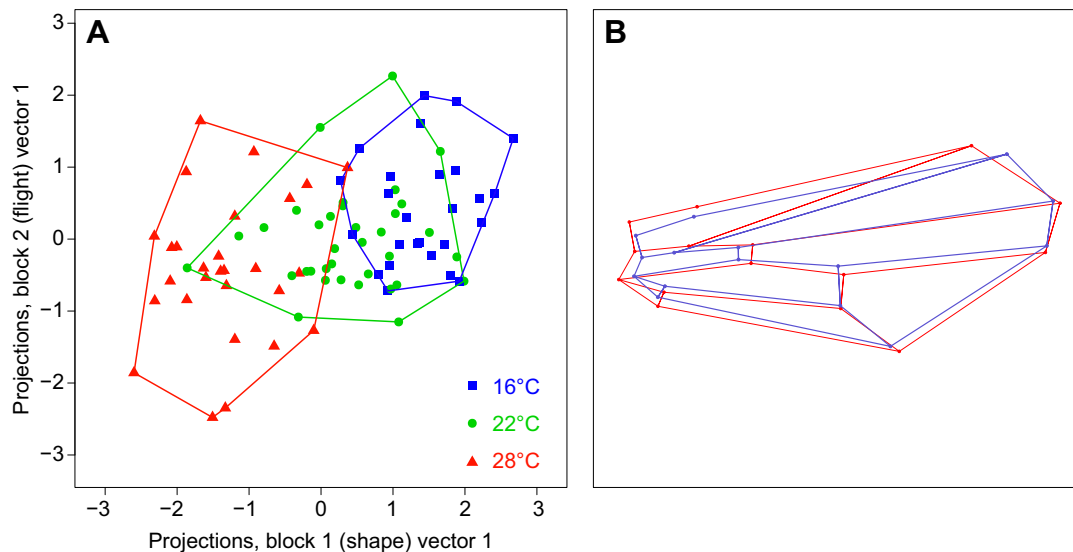
Fig. 4. Plot of the partial least squares (PLS) analysis. PLS plot (A) and the associated wing shape as described by the first PLS axis (B) are shown. The visualization plot corresponds to negative and positive values along the first PLS axis. For the sake of simplicity, extreme wing shapes are coloured according to the associated temperature (blue=16°C, red=28°C).

conceivable that the compensatory property of reduced wing loading would translate into increased flight performance when no challenge is experienced during flight (i.e. when flight power is not decreased by low flight temperature, at 21°C). The increased flight performance we observe for flies reared at 16°C may thus result from the compensatory effect of reduced wing loading at a non-challenging flight temperature. The question remains of whether shape variation induced by developmental temperature also affects flight parameters or if differences in flight can be explained by wing size variation alone (i.e. reduced wing loading).

Disentangling the effect of size from the effect of allometric changes induced by temperature was limited by the weak variation in size within temperature treatments. The covariation between wing shape and flight parameters (PLS analysis; Fig. 4) detected in our study is nevertheless compatible with the interesting hypothesis of an effect of shape variation on flight performance. This effect has often been assumed yet rarely tested in *Drosophila*, as wing morphology is usually described by univariate measure such as AR rather than multivariate shape data (but see Ray et al., 2016). Wing AR has been used to correlate wing shape and flight performance in various insect species, including *Drosophila* (Azevedo et al., 1998; Frazier et al., 2008). However, AR has been found to be of little aerodynamic importance compared with other descriptors of wing shape (Ellington, 1984; Usherwood and Ellington, 2002; Luo and Sun, 2005; Muijres et al., 2017). Moreover, different relationships between AR and temperature have been previously reported for different *Drosophila* species (negative in *D. melanogaster*: Azevedo et al., 1998; Frazier et al., 2008; positive in e.g. *D. buzzatii*: Bubliy and Loeschcke, 2005; see also Przybylska et al., 2016). Clear predictions on the effect of AR on flight performance thus seem difficult to formulate as they may not apply to *D. suzukii*. The overall absence of effect of rearing temperature on AR we describe here furthermore suggests that other components of wing shape may impact flight performance in cold-reared flies.

Specifically, the fastest, cold-reared, flies display wings with a contracted base and a slightly enlarged tip (i.e. narrower proximal section attached to the thorax and a distal shift of landmark 12; Fig. 2B). Consistently, rearing temperature impacted the distribution of the wing area along the wing axis as measured by

S2, with cold-reared flies showing higher S2 values. Such distal distribution of wing area has been shown to generate increased stroke force (Ellington, 1984). This shape change therefore matches the hypothesis of a compensation of a reduced wing beat frequency in the cold by an increased stroke efficiency. Increased S2 nevertheless comes at the price of an increased energetic demand (e.g. Outomuro et al., 2013). Investigating whether flight muscle mass is affected by developmental temperature would thus provide important complementary information in this context. Finally, landmark-based morphometrics allows quantification of the venation pattern. As structural components, veins contribute to the wing stiffness (or flexibility) and thus to how the wing will respond to the mechanical forces involved in flight (Wootton, 1992; Combes and Daniel, 2003). It is thus conceivable that the observed subtle changes in vein position induced by developmental temperature might alter wing deformability and thus, indirectly, aerodynamics.

Regarding the success of *D. suzukii*'s invasion, as we found no overall differences in wing shape and flight plasticities between native and invasive populations of *D. suzukii*, our results do not support the hypothesis of a role for wing plasticity to temperature in the success of the invasion. *Drosophila suzukii* populations used in this study were derived from similar latitudes within their respective countries (i.e. northern populations), with similar annual average temperatures. Relative climatic similarity between ranges could explain the absence of plasticity differences among populations as the colonization of the new range would not constitute an adaptive challenge for invading individuals.

In consideration of the foregoing, it is worth trying to put our experiments back into an ecological context (Arnold, 1983). *Drosophila suzukii* is considered to be a rather temperate species limited in its physiology by high temperatures and low humidity (Ometto et al., 2013; Tochen et al., 2014). Our lowest temperature of 16°C might not fall in the colder end of the temperature tolerance range for *D. suzukii*, especially for populations derived from northern latitudes. For instance, Dalton et al. (2011) investigated the seasonality of *D. suzukii* in Marion, Oregon (ca. 60 km from the sampling point of our US population). Their results indicate that *D. suzukii* activity, as described by trap counts, was higher in late September when the daily mean temperature was between 15 and

20°C. It is thus conceivable that, rather than testing cold versus warm treatments around an optimal physiological temperature of 22°C, we might have actually exposed cold-adapted *D. suzukii* to a range of somewhat unusually high temperatures. This could explain why our populations – all sampled from northern latitudes – showed increased speed and acceleration at 16°C compared with warmer temperatures. Additional work on the effect of temperature on flight would benefit from investigations of populations derived from the southern range of *D. suzukii* in the USA, Europe and Japan.

An explicit test for the adaptive nature of wing shape plasticity was beyond the reach of our experimental setup. The mere existence of plasticity does not imply anything about its adaptive value. Performance has been proposed as a bridge between phenotype and fitness in a series of influential papers (e.g. Arnold, 1983, 2003; Kingsolver and Huey, 2003; Wikelski and Romero, 2003 and references therein). A necessary – but not sufficient – condition to ascertain the selective value of a trait is that its variation should have functional consequences: a correlation between the morphological trait's value and performance is expected if that trait is important for fitness, whereas a lack of correlation would suggest neutrality. Here, we report a covariation between wing shape and flight. Furthermore, the expected effect of this shape variation on aerodynamic forces is congruent with the observed difference in flight performance. In conclusion, our results do not disqualify the adaptive hypothesis but rather encourage further investigation on the adaptive value of wing shape plasticity in relation to flight performance in *D. suzukii*, with additional attention to be given to flight temperature and populations derived from lower latitudes.

Supplementary information
Supplementary information available online at
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