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Temperature-induced phenotypic plasticity in the ovipositor of the invasive species *Drosophila suzukii*

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ABSTRACT

Drosophila suzukii (Matsumura, 1931) is a highly successful invasive dipteran which represents a serious threat for global fruit industry. Among other adaptive traits, *D. suzukii* owes its success to the derived morphological features of its ovipositor, which allows the insect to exploit the exclusive ecological niche of fresh fruit, thus avoiding competition with other closely related species. With the aim of investigating temperature-induced phenotypic plasticity of *D. suzukii* ovipositor, we reared this insect in four different laboratory conditions, represented by the combination of two developmental temperatures and two diet regimes for the larvae. We recorded the effects of these two factors on ovipositor size and shape and overall body size through a combination of distance-based and geometric morphometric analyses. Results showed that insects attain the largest body sizes at lower temperature, whereas the diet does not determine significant difference in size. However, the effect on size of the two factors is less pronounced in the ovipositor, which shows a negative allometry with respect to body size in all treatments. At higher temperature, ovipositor shape tends also to co-vary with its own size. Neither temperature nor diet have significant effect on ovipositor bilateral fluctuating asymmetry. These results confirm the hypothesis that in *D. suzukii* the toughened valve of the ovipositor are subjected to effective morpho-functional constraints, while probably being under strong selection by reason of their mechanical role.

1. Introduction

Temperature, probably the most pervasive and influential abiotic factors for life (Chown and Nicholson, 2004; Angilletta, 2009; Doucet et al., 2009) is a key driver of life history of species and their spatial distribution, and exerts large influence on population dynamics as well (Andrewartha and Birch, 1954; Cossins and Bowler, 1987; David et al., 1997; Angilletta et al., 2002; Bale et al., 2002; Hoffmann et al., 2003). At the organismal level, temperature governs all physiological processes, such as metabolic efficiency and growth rate, thus ultimately affecting the individual fitness (Chown and Nicolson, 2004; Angilletta, 2009).

Each organism and species is adapted to a specific temperature range, within which physiological processes are optimized and individual fitness is maximized. The width and the limits of this tolerance range are largely dependent on genetic traits, characteristic of the species and the individual. However, within these limits, and to a variable extent, organisms may respond plastically to temperature solicitations in dependence of the individual capacity for acclimation (Angilletta, 2009). Phenotypic plasticity, i.e. the capacity of a single genotype to be expressed as a range of phenotypes in response to different environmental conditions (Pigliucci, 2001; DeWitt and Scheiner, 2004) is an ubiquitous attribute of living organisms, and temperature is one of the main environmental factors able to triggering plastic responses (Fusco and Minelli, 2010).

The spotted wing drosophila, *Drosophila suzukii* (Matsumura, 1931) (Diptera, Drosophilidae), is a rapidly spreading invasive species which is creating massive damages to the small-fruit industry worldwide (Walsh et al., 2011; Cini et al., 2012; Rota-Stabelli et al., 2013). At variance with the vast majority of *Drosophila* species, which rely on rotting fruit and other decaying vegetal material for larval development, females of *D. suzukii* lay eggs in the flesh of ripening fruit, by puncturing their intact peel (Atallah et al., 2014). This ability is achieved through the exceptional features of the valves of the ovipositor, which appear strongly sclerotized and surmounted by a series of hard bristles in a "saw-like" structure (Lee et al., 2011; Atallah et al., 2014). The morphology of such toughened valves constitutes a "key innovation" of the species, i.e., the derived state of a character that confers significant adaptive advantage (Minelli and Fusco, 2005; Losos, 2010). Actually, it allows *D. suzukii* to exploit a new ecological niche, by

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laying egg on intact fruits that are inaccessible to other *Drosophila* species. Despite it is largely recognized that the specific features of *D. suzukii* ovipositor can effectively promote its adaptive success and invasive efficiency, little is known about the phenotypic plasticity of the morphology of this evolutionary novelty.

Temperature-induced phenotypic plasticity in *D. suzukii* has been recorded for different traits, such as the duration of postembryonic development, which is extremely rapid at high temperatures (Jakobs et al., 2015). In the field, plasticity was reported to produce two different seasonal phenotypes: a summer morph, characterized by lighter coloration and smaller size with respect to a winter morph, with a progressive increase in the frequency of the latter in the natural populations toward the end of the breeding season (Stephens et al., 2015; Shearer et al., 2016). Winter-morph phenotype could also be induced in laboratory, rearing the larvae at 10 °C (Shearer et al., 2016). However, other environmental factors may interact with the plastic response to temperature in affecting the magnitude or the direction of traits variation, as observed in *D. melanogaster*, where diet composition largely affect thermal tolerance (specifically, protein deficiency decrease the heat critical tolerance; Andersen et al., 2010).

In many organisms, the influence of temperature on the duration of development extends to the resultant adult body size. This has been described by the so called Temperature-Size Rule (TSR), which states that at higher temperatures, the development is faster and the final size is smaller, whereas at lower temperatures development is slower and animals reach larger size (Atkinson, 1994). The relationship between temperature and body size represents an classic case of phenotypic plasticity (Atkinson et al., 2006), however temperature can affect the size of different body parts either uniformly, without variation in body proportions, or differentially, producing more or less extensive shape changes (static allometry, Klingenberg and Zimmermann, 1992). With the present work we aimed at investigating temperature-induced phenotypic plasticity of *D. suzukii* ovipositor, and specifically whether its features are subjected to some morpho-functional constraints when flies are reared under different temperature in combination with distinct diet regimes. We found that, despite *D. suzukii* conforms to Temperature-Size Rule, possibly because of the largely recognized mechanical function of the toughened valves in oviposition, ovipositor size and shape are to a large extend conserved under different developmental conditions.

2. Materials and methods

2.1. Fly collection and establishment of experimental lines

Parental flies used to establish the experimental groups were obtained from laboratory meta-populations reared at 22 °C and originated from adults collected in fruit orchards (Tonina et al., 2016). Four experimental groups were established, combining two rearing temperatures (16 °C, *t16*, and 24 °C, *t24*) and two diets (standard, *d*+, and depleted, *d-*) resulting in 2 × 2 treatments (*t16d*+, *t16d-*, *t24d*+, *t24d-*). The standard diet (*d*+) consisted in a medium specific for *D. suzukii* rearing (Tonina et al., 2016). This is composed by raw cornmeal 75 g, dry-yeast 17 g, sucrose 15 g, soybean meal 12 g, agar 5.6 g, propionic acid 5 ml and water 1000 ml. All components are mixed and heated for 20 min at about 100 °C, except for propionic acid that is added at a temperature lower than 50 °C, just before pouring 40 ml of the medium in 120 ml urine container. The depleted diet (*d-*) medium was similar to *d*+, but contained one third of the amount of dry yeast and soybean meal, resulting similar in carbohydrates (90% of *d*+) and poorer in lipids and proteins (45% and 53% of *d*+, respectively). The two diets were implemented to mimic the nutritional resources that *D. suzukii* exploit in different seasons in the field (Bellamy et al., 2013; Tochen et al., 2014; Asplen et al., 2015), in particular to mimic a mix of cherry, blackberry and raspberry for *d*+ and a mix of grapefruit, blueberry, strawberry and elderberry for *d-* (see USDA, https://ndb.nal.usda.gov/ndb/search/list, 2016). Carbohydrate amount in ripe fruits is not considered a limiting factor for larval development of drosophilid flies (Reis, 2016; Little et al., 2017). The two diets allowed to assess how and to what extent the phenotypic response to different developmental temperatures can depend on nutritional conditions.

Each experimental group was reared in a net cage (25 × 25 × 25 cm) and provided with a container with the medium, essential for feeding, oviposition and larval development, and two vials containing tap water to maintain adequate humidity and watering. They were maintained under constant temperature regime (treatment temperature ± 0.5 °C), 70–80% relative humidity and 16 L:8D photoperiod in climatic incubators (type ST3, Pol-Eko-Aparatura, Wodzislaw Ślasky, Poland).

The medium containers were replaced every 2 or 4 days, depending on the treatment, *t24* or *t16*, respectively. Newly metamorphosed individuals (i.e., the new generation) of any treatment group were transferred to a new common net cage under the same (treatment-specific) conditions, while the adults of the past generation were suppressed and all the undamaged individuals stocked in 75% ethanol. Two generations were produced for *t16d*+ and *t16d-* and four generations for *t24d*+ and *t24d-*. From the collection of stocked individuals, 113 females (29 *t16d-*, 30 *t16d*+, 24 *t24d-* and 30 *t24d*+) were subsequently used for morphometric analyses.

2.2. Ovipositor preparation for microscopy and image acquisition

Before dissection, females were left in distilled water for 24 h to ensure a complete rehydration. The insects were placed on their left sides and the thorax length was measured from the humeral callus to the posterior tip of the postscutum, under a stereomicroscope (Leica) at 160×, equipped with an eyepiece micrometer (div. 0.055 mm). Thorax length is usually considered a reference measure for the total body length in *Drosophila* (Partridge and Fowler, 1993).

With micropins and Dumont tweezers, the ovipositor was detached from the rest of the abdomen, and the two valves were separated and cleaned from the numerous membranes. A semi-permanent slide was mounted with both the valves of each individual, imbedded in Faure's liquid and covered with a circular coverslip.

The valves were photographed under a light microscope (Carl Zeiss Jena), at 500×, using a camera Reflex Pentax-kr in automatic mode, acquiring images at a size of 4288 × 2848 pixels.

2.3. Landmark choice and digitalization

A set of four landmarks and three semilandmarks for each ovipositor valve was set up along the valve profile (Fig. 1). Two landmarks were positioned at distal and proximal tips of the valve (lmks. 1 and 4, respectively), one between the second and the third ventral bristles (lmk.

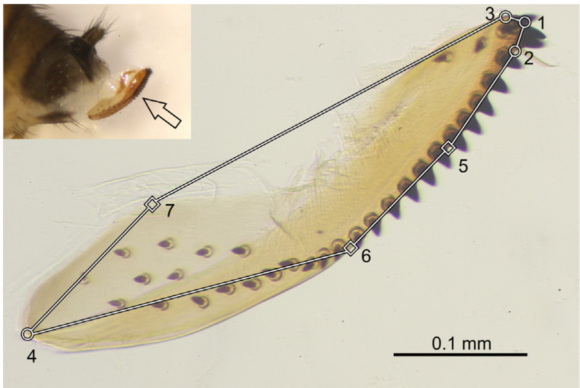

Fig. 1. Position of landmarks (circles) and semilandmarks (diamonds) on the left valve of *D. suzukii* ovipositor.

2) and one between the first and the second dorsal bristles (lmk. 3). One semilandmark was positioned between the seventh and the eighth ventral bristles (lmk. 5), one between the thirteenth and the fourteenth ventral bristles (lmk. 6) and one at the extremity of the proximal rounded protrusion of the dorsal edge (lmk. 7).

Semilandmarks provide a way for capturing size and shape information on body regions that do not include well-defined homologous features (Gunz and Mitteroecker, 2013), as for instance along curved edges. However, as semilandmarks, at variance with landmarks, are not point locations that are biologically homologous among specimens, many morphometricians suggest keeping their number at a minimum and recommend some caution in interpreting the results (see Cardini and Loy, 2013). The three semilandmarks we used is the smallest number that allows not to completely overlook the geometric information contained in the curved profiles of the dorsal and ventral edges of the valve.

Images were loaded and scaled in TPSDig 2 (ver. 2.26; Rohlf, 2015). Landmarks and semilandmarks were digitized twice on each image by the same person (MC) in different days, in order to quantify and minimize the measurement error due to digitalization (Palmer, 1994; Klingenberg and McIntyre, 1998). Then, semilandmark coordinates were allowed to slide along the profile using the Minimum Squared Procrustes Distance criterion in TPSRelw (ver. 1.65; Rohlf, 2015), which increases the geometric correspondence of these special points. In all successive analyses, semilandmarks and landmarks were treated in the same, so that, from now on, we will refer to both as 'landmarks'.

2.4. *Geometric morphometrics and statistical analyses*

Ovipositor size and shape were analyzed using the methods of geometric morphometrics, that are based on the use of landmarks. Configuration of raw coordinates of these points were translated, scaled and rotated through a Procrustes generalized least square superimposition, to obtain a standard measure of size and scale-independent shape coordinates (Adams et al., 2004; Cardini, 2013). Size was estimated as the centroid size (CS), the square root of the sum of squared distances between each landmark and the centroid of the configuration (Bookstein, 1991). When not stated otherwise, morphometric analyses were executed using the software MorphoJ (ver. 1.06d; Klingenberg, 2011).

The ovipositor is composed of two paired structures, the valves, which exhibit a type of bilateral symmetry generally referred to as *matching symmetry* (Klingenberg et al., 2002; Klingenberg, 2015). Procrustes methods allow to partition symmetric and asymmetric components of shape variation.

The effects of several factors on ovipositor variation in size and shape were investigated using a Procrustes analysis of variance (Procrustes ANOVA, Klingenberg et al., 2002) on the dataset including all the four treatments. Total shape variation was decomposed into the main effect of 'individual' (i.e., variation among individuals), 'side' (i.e., directional asymmetry between the two valves), the interaction 'individual-by-side' (i.e., fluctuating asymmetry between the two valves) and measurement error due to digitizing (Palmer and Strobeck, 1986; Klingenberg et al., 2002). Two 'extra effects' represented by diet and temperature were also included.

To study the influence of temperature on ovipositor size, we first carried out univariate ANOVAs on Thorax size and ovipositor CS, followed by Tukey's pairwise tests using the software Past.exe (ver. 3.14; Hammer et al., 2001). After that, we performed an allometric analysis of ovipositor proportions using thorax size as proxy for body size, separately for the four datasets corresponding to the four treatments. Individual ovipositor CS was obtained averaging the measures in the two valves and in the two digitalizations, and both Thorax Size and ovipositor CS were transformed to their natural logarithms for the following linear regression analysis. Significance of the allometric relationships (difference from 1) and differences among allometric relationships in the four treatments were tested with Student's *t*-test on the regression coefficients.

The influence of temperature on ovipositor shape was investigated through multivariate regressions of ovipositor shape onto its size (log-transformed CS) on the dataset averaged by side and digitalization. Significance of the regression was assessed with a permutation test (10,000 rounds). On the same dataset, we also carried out a canonical variates analysis to test for shape differences between the four treatments. Significance of the differences was calculated with a permutation test (10,000 rounds).

Finally, we searched for difference in size and shape bilateral fluctuating asymmetry (FA) between the four treatments. We repeated the Procrustes ANOVA (see above) separately for each treatment and calculated the FA10 index of Palmer and Strobeck (2003) as 0.798 (MSsj – MSm), where MSsj is the individual-by-side mean squares and MSm is the measurement error mean squares. Because FA10 is a variance estimate, we used Fisher's tests to evaluate differences in FA between treatments. All FA calculations were implemented in an Excel spreadsheet.

3. Results

3.1. *Effects of diet and temperature on size and shape variation*

Procrustes ANOVAs of size and shape (Table 1) show that all the effects are significant at the conventional level of $P < 0.05$, to the exclusion of diet for size. Individual variation accounts for 15% of the size total sum of squares and for 57% of the shape total sum of squares. The effect of diet is non-significant for size ($P = 0.08$) and modest for shape (1% of total sum of squares; $P = 0.0017$). On the contrary, temperature shows a strong influence on size, explaining 83% of the total sum of squares, and a minor influence on shape, accounting for 9% of the total shape sum of squares. Individual-by-side effect (FA, fluctuating asymmetry) accounts only for 1% of the size total sum of squares, and for 28% of shape total sum of squares.

3.2. *Influence of temperature on ovipositor size*

Both thorax and ovipositor were significantly larger in treatment groups *t16* than in *t24*, whereas the two diets did not determine significant difference in sizes (Tukey's test, Table 2).

The analysis of the relationships between thorax and ovipositor size

Table 1

Variation of ovipositor size and shape from Procrustes ANOVAs on *D. suzukii* samples reared at different diet and temperature conditions. SS, sum of squares; SS%, percentage of total sum of squares; MS, mean squares; Df, degrees of freedom; F, Fisher's test value; *P*, p-value.

SIZE

Effect	SS	SS%	MS	Df	F	*P*
Individual	0.0573	15.05	0.0005	114	14.87	< 0.0001
Side	0.0005	0.13	0.0005	1	15.16	0.0002
FA	0.0039	1.03	3.4E-05	116	92.35	< 0.0001
Temperature	0.3171	83.35	0.3171	1	631.16	< 0.0001
Diet	0.0015	0.41	0.0015	1	3.08	0.0817
Error	0.0001	0.02	3.7E-07	234	–	–

SHAPE

Effect	SS	SS%	MS	Df	F	*P*
Individual	0.1137	57.16	0.0001	1140	2.10	< 0.0001
Side	0.0068	0.13	0.0007	10	14.40	< 0.0001
FA	0.0550	27.64	4.7E-05	1160	51.14	< 0.0001
Temperature	0.0184	9.26	0.0018	10	18.46	< 0.0001
Diet	0.0028	1.42	0.0003	10	2.84	0.0017
Error	0.0022	1.09	9.3E-07	2340	–	–

Table 2
Tukey's Q, significance and F-values from Univariate Analysis of Variance on thorax and valves size of *D. suzukii* samples reared at different temperature and diet conditions.

	Thorax			Ovipositor		
	t16d-	*t16d+*	*t24d-*	*t16d-*	*t16d+*	*t24d-*
t16d+	0.35^{ns}			2.73^{ns}		
t24d-	14.11^{*}	13.76^{*}		24.29^{*}	27.02^{*}	
t24d+	16.09^{*}	15.74^{*}	1.98^{ns}	23.09^{*}	25.82^{*}	1.20^{ns}
	$F_{3,113} = 75.32$; $P < 0.0001$			$F_{3,113} = 211$; $P < 0.0001$		

ns, non significant.
* $P < 0.001$.

showed negative allometry under all treatments (Fig. 2), with the extreme case of treatment *t16d+*, where ovipositor size did not vary significantly with thorax size (non-significant regression coefficient). With respect to this treatment, both diet and temperature (and their combination) showed to be effective in influencing valve allometry (*t16d+* vs. *t16d-*, *t24d+*, *t24d-*), producing significantly higher regression coefficients (Student's *t*-test on regression coefficient differences, all *P-values* < 0.018).

3.3. Influence of temperature on ovipositor shape

The analysis of multivariate regressions between valve size and shape showed that at both diet regimes there was a common pattern of ovipositor shape variation with size (ovipositor CS). At higher temperature (*t24*), appreciable variation of shape with size was observed (although significant only under depleted diet *t24d-*, permutation test, 10,000 permutation rounds, $P < 0.0001$), whereas at lower temperature (*t16*) at both diet regimes, ovipositor shape did not change visibly with size (*t16d+*; *t16d-*; permutation test, 10,000 permutation rounds, $P > 0.01$). Overall, ovipositor shape co-varies with size (permutation test, 10,000 permutation rounds, $P < 0.0001$), ranging from relatively dorso-ventrally expanded at smaller sizes to relatively dorso-ventrally narrowed at larger sizes (Fig. 3).

Canonical variates analysis revealed highly significant pairwise shape differences between the four treatments ($P < 0.001$), but for the comparison between the two *t16* diets that resulted significant to a lesser degree ($P = 0.0374$, Fig. 4). Shape along the first canonical variate, which explains 86% of the between-treatments shape variance, ranges from an ovipositor distally expanded dorso-ventrally to an ovipositor with closer dorsal and ventral distal edges, the major effect being produced by the temperature. The second canonical variate (9% of the variance) ranges from an ovipositor shape medially dorso-ventrally expanded to a shape medially narrower.

3.4. Influence of diet and temperature on ovipositor size and shape FA

Fluctuating asymmetry was significant for both size and shape (Table 1). However, FA10 indexes did not reveal a consistent pattern of FA differences among the four treatments. Out of the 12 pairwise comparisons (six for size and six for shape), only two resulted significant ($P < 0.05$). Temperature affected shape FA only at the depleted diet regime (*t16d-* vs. *t24d-*) and diet affected shape FA only at 16 °C (*t16d-* vs. *t16d+*).

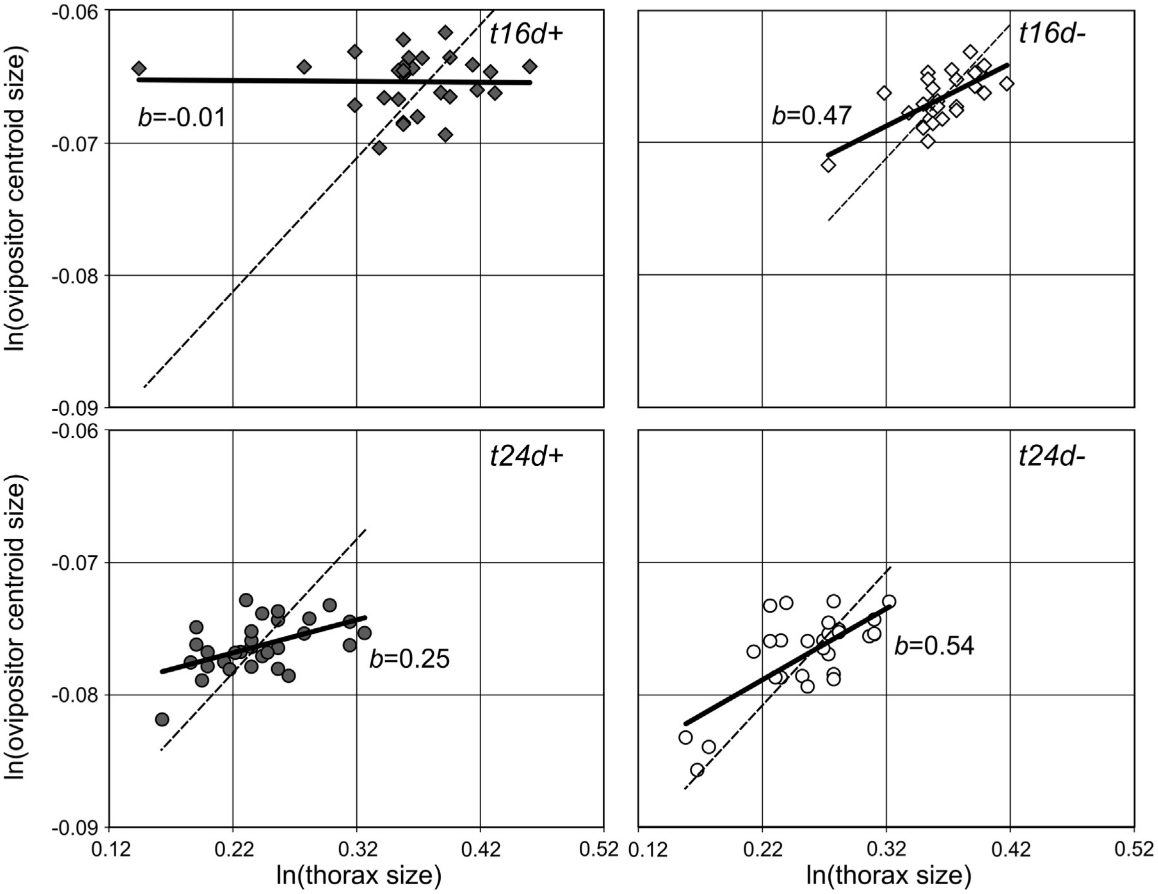

Fig. 2. Allometric relationships between log-transformed thorax size (mm) and ovipositor valve size (CS) in the four treatment groups. Solid lines: regression lines; dashed lines: isometry lines. All allometric coefficients (*b*) are significantly smaller than 1 (isometry), indicating negative allometry. In *t16d+* coefficient *b* does not differ significantly from 0 (variable independence).

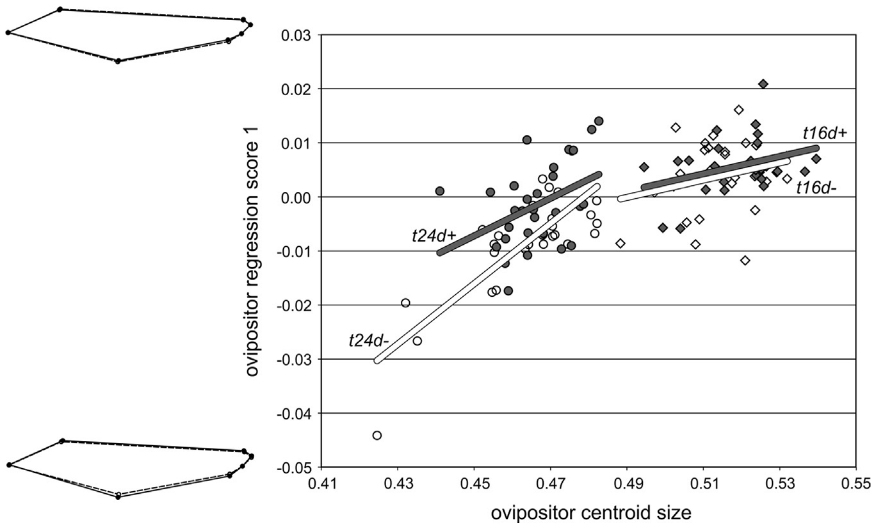

Fig. 3. Multivariate regressions of ovipositor valve shape (Procrustes coordinates) on valve size (CS). Filled diamonds: *t16d+* treatment; empty diamonds: *t16d-* treatment; filled circles: *t24d+* treatment; empty circles: *t24d-* treatment.

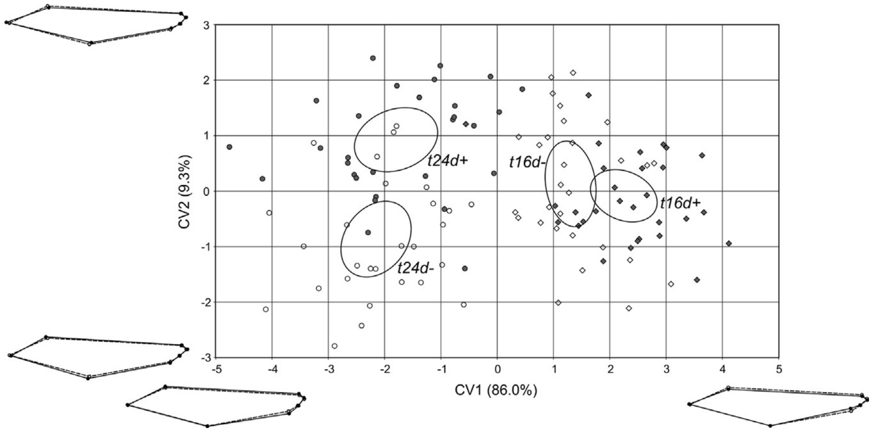

Fig. 4. Differences between the four treatment groups in relation to the first two variates from canonical variates analysis on the ovipositor valves. Shape variation along the two axes is shown as solid line wire frames with respect to the average shape (dashed line wire frames). Filled diamonds: *t16d+* treatment; empty diamonds: *t16d-* treatment; filled circles: *t24d+* treatment; empty circles: *t24d-* treatment.

4. Discussion

We found significant plastic effects of the developmental temperature on several morphological traits in adult female *D. suzukii.* These include body size, ovipositor size and shape, and ovipositor allometry.

Temperature has a significant effect on the whole body size and the ovipositor size alike. This was observed at both diet regimes, which per se do not significantly affect the size of the insects. The experiment confirmed the validity of TSR for thorax size and, to a lesser extent, for ovipositor size as well, with individuals reared at 16 °C being significantly larger than those reared at 24 °C (Fig. 2). This is consistent with the fact that at lower temperatures development is slower (about 20 days rather than 30, with standard diet), and in arthropods this typically results in larger body sizes (Roff, 2002; DeLong and Hanley, 2013). In addition, despite the design of our experiment did not include extremely cold conditions (10 °C), our results are consistent with the observation of larger size *D. suzukii* adult phenotypes at low temperatures, as it occur in autumn and winter (Shearer et al., 2016).

Despite conformity to TSR, there is a consistent negative allometric relationship among ovipositor size and body size for all treatments (Fig. 2). In other words, ovipositor size tends to vary among individuals to a much lesser degree than body size, suggesting a more strict size control for this key structure under all developmental conditions. Notably, at *t16d+* ovipositor size does not vary with body size at all, showing an even higher effectiveness of developmental size control under the most favorable rearing conditions. This means that although diet per se has no effect on either the mean body size or the mean ovipositor size, nonetheless diet in combination with temperature can affect the within treatment relationship between body size and ovipositor size. These results confirm the hypothesis that in *D. suzukii* the toughened valve of the ovipositor are subjected to effective morpho-functional constraints, while probably being under selection by reason of their mechanical function. These findings are in agreement with Eberhard's (2009) survey on a large sample of arthropod and vertebrate specie, showing that external genital structures tend to exhibit negative static allomety, with lower coefficients than other body parts, which is explained by both natural selection and sexual selection. In the specific case of *D. suzukii* ovipositor, one can speculate about a potential trade-off between natural selection, that would favor more robust ovipositor valves to more effectively perforate the fruit skin (Atallah et al., 2014), and sexual selection, where mechanical and sensorial match with male genitalia would favor intermediate-sized female genitalia (Eberhard, 2009).

In insect, variation in size of a given structure may be not paralleled by shape variation of the same magnitude (Eberhard, 2009), as in the case of the scarabeid beetle *Phyllophaga hirticula*, where both male and female genitals are characterized by low intraspecific variation in size but high shape variation (Polihronakis, 2006). In *D. suzukii* the developmental temperature modestly affects ovipositor shape, whereas the influence on ovipositor size is significantly larger (Table 1). This is consistent with the idea that the shape of the ovipositor is subject to either or both stronger constraints and stronger selections, suggesting that it is this aspect of ovipositor morphology that has a major role as key innovation for *D. suzukii* evolutionary success. Actually, both size and number of *D. suzukii* ovipositor strong bristles are comparable to that of the sister species *D. subpulchrella*, but the latter, because of a stouter ovipositor overall shape, cannot penetrate fresh fruit skin with the same effectiveness of *D. suzukii* (Atallah et al., 2014).

The shape of ovipositor valves also co-varies with their size, showing a distinctive allometric pattern (Fig. 3). The effect of size on shape is more pronounced at higher temperature, irrespective of the diet regime. Hence, higher temperature not only determines ovipositors of a smaller size, but it also affects other aspects of valve morphogenesis which result in a higher degree of shape variation. This is perhaps an effect of a more efficient shape control at lower temperature, when development is slower, a form of developmental stability not detected by FA (see below).

Finally, we tested the effect of temperature on ovipositor developmental stability, as it appears from the analysis of fluctuating asymmetries (FA; Palmer and Strobeck, 1986). Developmental stability is the property of an organism to buffer random perturbations of the developmental process, and random non heritable deviations from bilateral symmetry, or FA, have been largely employed to investigate levels and patterns of developmental stability in many taxa (Møller and Swaddle, 1997; Polak, 2003; Klingenberg, 2015). Developmental instability can be produced by a variety of stressful environmental factors, among which poor food quality or unsuitable developmental temperature. As a side-effect of the plastic response of *D. suzukii* to poorer diet and higher temperature, we checked for consistent differences in the level of ovipositor FA among the treatments and we found none, neither for size, nor for shape. The two environmental variables, within the limits of the tested values, while producing a plastic response in the ovipositor size and shape, do not significantly affect at least some of the buffering capacities of developmental systems of the insect.

In conclusion, this study on the temperature-induced phenotypic plasticity in *D. suzukii* showed how this can be differently modulated in distinct phenotypic traits. In particular, it emerges a tight developmental control of both size and shape of the ovipositor valves, which might be related to the specific mechanical function of these structures.

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