Entomological Research

Entomological Research **48** (2018) 262–268

RESEARCH PAPER

Effect of fruit host on wing morphology in *Drosophila suzukii* (Diptera: Drosophilidae): A first view using geometric morphometrics

Ivana PAJAČ ŽIVKOVIĆ[1], Darija LEMIC[1], Aleksandar MEŠIĆ[1], Božena BARIĆ[1], Rodrigo ÓRDENES[2] and Hugo A. BENÍTEZ[2,3]

[1] Department of Agricultural Zoology, University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000, Zagreb, Croatia
[2] Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile
[3] Museum of Zoology, Cambridge University, Cambridge, UK

Correspondence
Hugo A. Benítez, Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas Universidad de Tarapacá, Arica y Parinacota 1000000 Arica, Chile.
Email: hbenitez@uta.cl

Received 29 May 2017;
accepted 18 October 2017.

doi: 10.1111/1748-5967.12278

Abstract

The invasive alien fruit pest *Drosophila suzukii*, (Matsumura 1931) causes economic loss in soft-skinned fruit production across Europe. After its first detection in 2008, the species has successfully expanded to a wide geographic area and invaded new host plants in a relatively short period of time. The aim of the present study was to analyze the connection between food preferences as host specialization and the morphology of *D. suzukii*. Population morphological variation in wings was investigated in two different host fruits (grape and strawberry) in which economic damage has been recorded. The geometric morphometric results revealed two noticeable wing shape morphotypes in *D. suzukii* (i.e. vein configuration) between the grape and strawberry fruits. Flies reared in grapes had wider wings, whereas flies grown in strawberries had more narrow wings. These differences in morphotype could be explained by the effects of wing aerodynamics, which affect the strength of the wings in flight. This, in turn, can lead to better dispersion within the associated fruit host. These results confirm that this extremely invasive species, found worldwide, is successful at spreading in part because of its potential to adapt rapidly under different rearing conditions. Therefore, adaptive variations in the wing shape of *D. suzukii* can be used to differentiate populations based on food preference (e.g. soft fruits) and can serve as an additional tool for detecting different bioecological types of *D. suzukii*.

Key words: fruit pest, geometric morphometrics, spotted wing *Drosophila*, wing shape.

Introduction

The spotted wing drosophila (*Drosophila suzukii* (Matsumura 1931)) is a highly polyphagous invasive species of south-east Asian origin (Cini *et al*. 2014). The species is extremely fertile and has a wide range of host plants and high dispersal potential, and is therefore characterized as a pest of great concern in Europe (Cini *et al*. 2012; Asplen *et al*. 2015). The pest was first registered in Europe in 2008 (Calabria *et al*. 2012; Cini *et al*. 2014) and damage was found during 2009 in fruit production in Italy (Grassi *et al*. 2009). Unlike most other Drosophilidae, *D. suzukii* can lay eggs in healthy, unwounded ripening soft-skinned fruit, including grapes (*Vitis vinifera* L.) (Seljak 2011). In recent years, severe economic damage in European fruit production has been observed (Cini *et al*. 2012).

In Croatia, *D. suzukii* was first reported in 2010 in the eastern part of the country (Istria County) on raspberry (*Rubus idaeus* L. 1753), peach (*Prunus persica* (L.) Batsch 1801) and grapes. Since then, *D. suzukii* has spread across the coastal region and into northern counties

(Masten Milek *et al*. 2011; Bjeliš *et al*. 2014b). Host plants for *D. suzukii* in Croatia include cherries (*Prunus cerasus* L. 1753 and *Prunus avium* L. 1755), peach, apricot, nectarine, plum, berries (*Rubus* spp. and *Fragaria* spp.), fig, and grapevine (*Ficus* spp. and *Vitis* spp.) (Masten Milek *et al*. 2015). In 2016, the first economic damage in strawberry (Mešić *et al*. 2017) and grape (Mešić *et al*., unpubl. obs., 2016) production was observed. Today, *D. suzukii* is established throughout Croatia, and therefore Croatian fruit production is under serious threat from this species.

Various environmental conditions result in different levels of stress for insects, which affects the ability of individuals to adapt and spread (Clarke 1998; Lalouette *et al*. 2011). The adaptation over time for a species to a specific environment is the result of environmental influence, pressure, and geographic distance (Alibert *et al*. 2001). Adaptive variation in insects reflects the historical evolution, and determines a population's phenotypic response (Ghalambor *et al*. 2007; Pfennig *et al*. 2010; Parsons & Joern 2014). Morphology can be studied by analyzing morphometric measurements (i.e. shape and size) and it's changes in relation to other factors (e.g. host and growing conditions; Rohlf & Marcus 1993; Adams & Rohlf 2000; O'Higgins 2000; Henderson 2006). Studies have analyzed the effects of environmental conditions on insect morphology, obtaining important information about the effects of ecological factors using geometric morphometry (GM; Benítez *et al*. 2014a, 2014b; Lemic *et al*. 2016; Mikac *et al*. 2016; Lunardi *et al*. 2017). These studies have provided evidence of developmental instability, as well as differences between biotypes regarding environmental conditions (soil type, climate and nutrition; Lemic *et al*. 2014; Benítez *et al*. 2014a, 2014b). GM methods can also be used as a tool to assess the occurrence and expansion of certain insect species, especially invasive species (Benítez *et al*. 2014a, 2014b; Mikac *et al*. 2016). Similarly, GM techniques based on wing shape analyses represent an approach for discriminating between morphologically cryptic taxa, such as shown by Schutze *et al*. (2012) for the *Bactrocera dorsalis* species complex.

During their colonizing processes, invasive species (such as *D. suzukii*) show adaptive clinical variation, indicating that new environmental conditions (selective factors) have favored rapid evolution (Gilchrist & Huey 2004).

Therefore, the aim of the present study was to analyze the association between environmental factors, such as host specialization, and *D. suzukii* morphology. By understanding this relationship, we can provide better insights into how this species adapts morphologically to new rearing conditions. This evidence could, in turn, provide useful information about the invasive processes of this species.

Materials and methods

Sample sites and data acquisition

Flies were collected in 2016 (from 6 September to 6 October) in two different semidomestic habitats in Croatia in which economic damage to fruit growing has been observed. The first habitat was an Integrated Pest Management (IPM) vineyard (46°28′1.2″N, 16°24′0″E) situated in the northern part of Croatia (Medjimurje County). Two grape (*Vitis vinifera* L.) cultivars ('Sauvignon blanc' and 'Riesling') are grown in this vineyard. The site is surrounded by forest and an extensive production orchard. The second habitat was an IPM commercial strawberry farm (45°41′ 24″N, 16°24′0″E) situated in central Croatia (Zagreb County). Three strawberry (*Fragaria* × *ananassa* Duch.) cultivars ('Albion', 'Portola', and 'San Andreas') are grown under greenhouse conditions, with administrative buildings and houses surround the site. The flies were sampled using an apple vinegar trap and preserved in 70% ethanol prior to determination. The species and sex identification of *D. suzukii* was conducted according to the recommendations of European and Mediterranean Plant Protection Organization (OEPP/EPPO) (2013). In all, 200 adult *D. suzukii* were collected (100 per location; 50 males, 50 females). Left and right wings were removed from each individual and slide mounted using the fixing agent Euparal (Australian Entomological Supplies, Melbourne, Vic., Australia) based on standard methods (Upton & Mantel 2010) for subsequent GM analysis. Slide-mounted wings were photographed using a Leica (Wetzlar, Germany) DFC295 digital camera (3 megapixel) on a trinocular mount of a Leica MZ16a stereo-microscope and saved in JPEG format using the Leica Application Suite version 3.8.0 (Leica Microsystems, Australia). Fifteen landmarks (Fig. 1) defined by vein junctions or vein terminations (Bookstein 1991) were digitized using the software ImageJ 1.50i (National Institutes of Health, Bethesda, MD, USA).

Multivariate analyses of size and shape

To extract wing shape data, a full Procrustes fit was used. Procrustes superimposition is a procedure that removes the information of size, position, and orientation to obtain morphometric variables (Rohlf & Slice 1990). A measurement error analysis was performed to corroborate that the results corresponded to real shape changes and not digitizing error. This error was calculated after digitizing a sample of pictures twice and comparing the individual and errors of the mean square (MS) values from the Procustes analysis of variance (ANOVA). To quantify the shape variation related to the different host fruit, a principal component analysis (PCA; Jolliffe 2002) was performed. To evaluate whether size could affect the shape variation between host as an allometric effect, multivariate

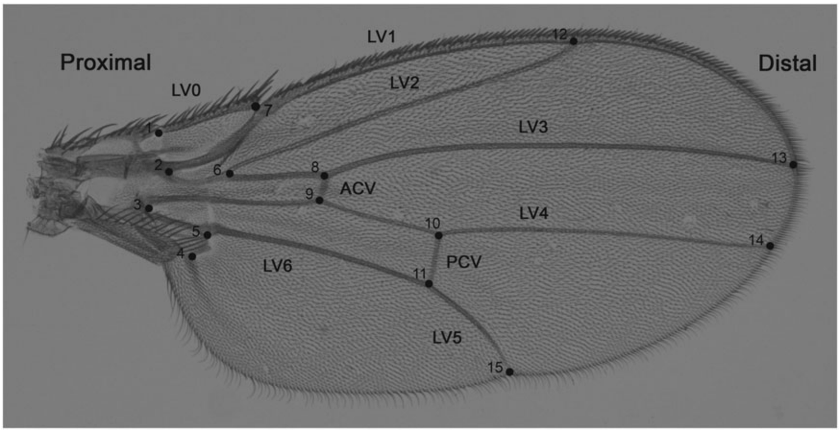

Figure 1 Ventral view of *Drosophila suzukii* wing morphology with the 15 landmarks used to characterize its shape. LV, longitudinal vein; PCV, posterior crossvein; ACV, anterior crossvein.

regression of shape versus centroid size was performed (Monteiro 1999). Finally, two-way ANOVA using the factors of fruit host and sex was performed using the shape variables to identify significant differences. All the aforementioned analyses were performed in MorphoJ v1.05d (available for free under the Apache License, Version 2.0. // http://www.flywings.org.uk/morphoj_page.htm) (Klingenberg 2011).

Results

A Procustes ANOVA was performed after combining two independent measurements of the samples, revealing that values of the MS of the error are smaller than the individual × size (Table 1) discarding measurement error of the landmarking process.

The PCA (Fig. 2) showed that the first three components accounted for 69.8% of the total shape variation in *D. suzukii*, with a clear differentiation in the shape variation of the first dimension (PC1: 46.7%; PC2: 12.8%; PC3: 9.5%). In a detailed view of the shape variation, the results showed that the main differentiation is related to flies reared in different fruit hosts (Fig. 3). A noticeable expansion was identified in the radial vein Landmark #12 for flies reared in grapes. In addition, in the anterior section of the wing, a widening contraction of Landmarks #2 and #6 differentiated the wings of grape- and strawberry-reared flies, with the wing is noticeably thinner with a slight contracted radial veins. There was little difference in all other landmarks in flies reared in grapes versus strawberries.

A multivariate regression was performed to analyze the allometric effect of the samples. A significant effect was found, but only 8.43% was explained by allometry, and this was mostly found in flies reared in grapes (Fig. 4). Finally, the two-way ANOVA found significant differences using the shape and centroid size between hosts, and sexual dimorphism analysis revealed only differences in shape and not in centroid size (Table 1).

Table 1 Procrustes analysis of variance (ANOVA) for both centroid size and shape of *Drosophila suzukii*, characterized by matching symmetry

Effect	SS	MS	d.f.	*F*-value	*P*-value	Pillai tr.	$P_{parameter}$
Centroid size							
Host fruit	1.233301	1.233301	1	12.84	0.0004		
Sex	0.654578	0.654578	1	6.81	0.0097		
Individual	18.92189	0.09605	197	52.56	<0.0001		
Side (left/right)	0.000402	0.000402	1	0.22	0.6394		
Individual × side	0.363665	0.001827	199	1.26	0.0282		
Error 1	0.579354	0.001452	399				
Shape (Wing shape)							
Host Fruit	0.10205846	0.003925325	26	102.4	<0.0001	0.88	<0.0001
Sex	0.00607654	0.000233713	26	6.1	<0.0001	0.63	<0.0001
Individual	0.19636363	3.83373E-05	5122	6.77	<0.0001	19.66	<0.0001
Side (left/right)	0.0004789	1.84192E-05	26	3.25	<0.0001	0.31	<0.0001
Individual × side	0.029284	5.6598E-06	5174	11.26	<0.0001	19.57	<0.0001
Error 1	0.00521569	5.028E-07	10374				

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). Pillai's TracePillai's is one of several test statistics used in MANOVA.

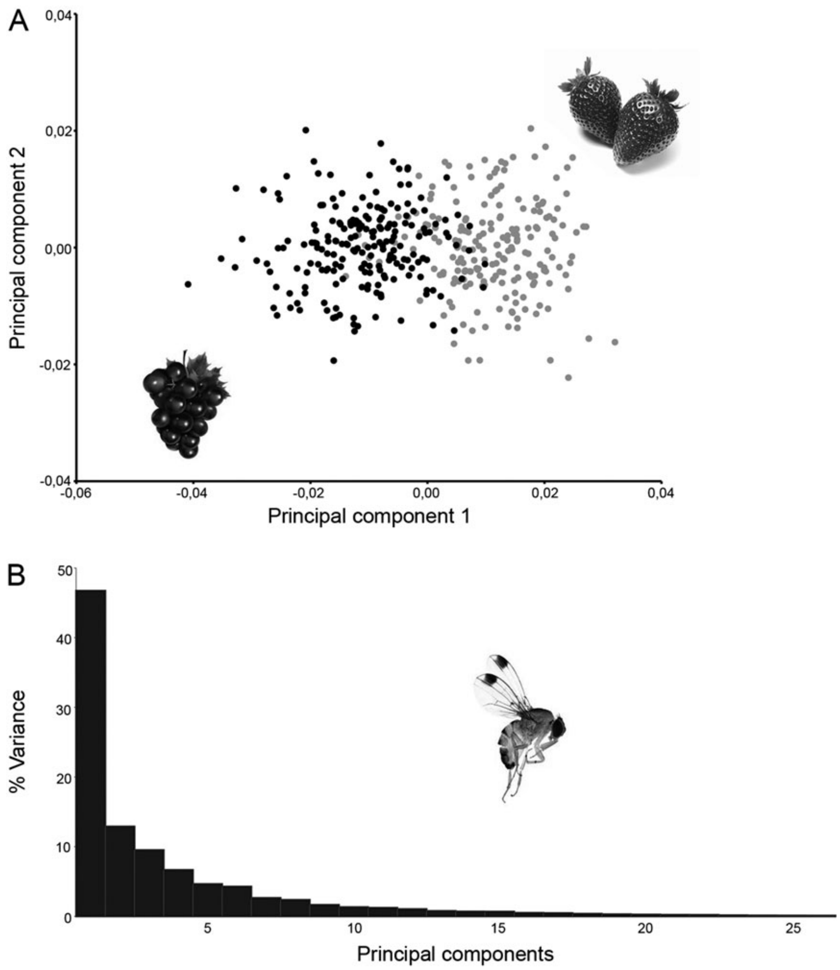

Figure 2 Principal component analysis for the *Drosophila suzukii* wing shape. (A) Scatterplot of the first two principal components used to display most of the variation of shape. The gray and black dots represent the wing shape of individuals growing in strawberries and grapes, respectively. (B) Histogram of the percentage shape variation by dimensions in the shape space.

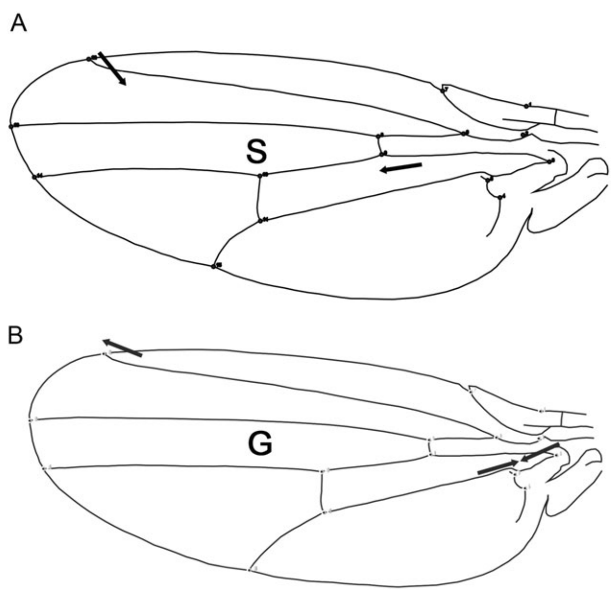

Figure 3 Wing shape variation between flies in different fruit hosts: (A) strawberry; (B) grape. The arrows represent the vector movement between samples.

Discussion

The present study is the first to focus on *D. suzukii* using GM methods. Previous studies of differences in insect shape showed that GM can be used in pest population studies (Mikac *et al*. 2013; Benítez *et al*. 2014a, 2014b; Lemic *et al*. 2014). Conditions prevailing during larvae development can cause morphometric differences in adult shape (Benítez *et al*. 2014b). In the present study, the effect of the host (food preference) on *D. suzukii* wing shape was assessed. The results demonstrate the plasticity of wing morphology in *D. suzukii* after being reared in two different host fruits. The GM results found two noticeable wing shape morphotypes (vein configuration) after a comparison of the average shape of wings in flies reared in grape and strawberry. It is known that the wing venation pattern of insects acts as a passive support, but also controls the three-dimensional form of the wing (Wootton 1981). The first principal component (PC1; simulation of morphospace in GM) shows a conspicuous variation of the wing in *D. suzukii*, mediated by the vector movement of the landmarks. This variation is located at radial

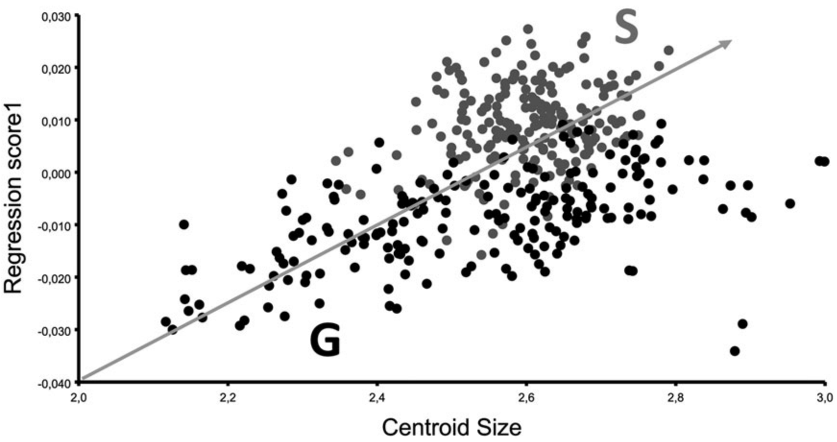

Figure 4 Multivariate regression analysis of *Drosophila suzukii* wing shape showing the relationship between wing size and shape in flies for both fruit hosts: (S) strawberry; (G) grape. Of note is the the large distribution of sizes in the flies growing in grapes.

veins and the set of veins located at the anterior section of the wing: narrow wings in flies grown in strawberries and wider wings in for flies grown in grapes (Fig. 3). These specific morphotypes could affect the wing aerodynamics, which may affect the strength of the wing in flight (Dudley 2002), and therefore contribute towards better dispersion and to success as a wide-ranging invasive pest.

The use of GM to study host effects is linked with tools of fluctuating asymmetry that analyse the pattern of left and right variations as products of developmental instability (Polak 2003). Most studies use laboratory experiments to identify developmental changes in the morphology of an organism (Hoffmann *et al*. 2005; Carreira *et al*. 2008; Soto *et al*. 2008, 2010). However, GM has also been used to measure insect wing shape changes in response to environmental variables (Johansson *et al*. 2009; Benitez *et al*. 2014b; Lemic *et al*. 2016) or sexual dimorphism (Adams & Funk 1997; Valenzuela *et al*. 2004; Gidaszewski *et al*. 2009; Benítez *et al*. 2011, 2013; Siomava *et al*. 2017).

In Croatia, as a relatively recent invader, *D. suzukii* has adapted to different living conditions in the areas investigated in a short time since its first detection in 2010. The results of the present study confirm that this extremely invasive species is successful worldwide because of its potential to adapt in different rearing conditions. Previous studies have shown that temperatures, adverse nutritional stress, and many other factors that generate stress during development can lead to increased morphological differences (Clarke 1998; Benítez *et al*. 2008, 2013; Lemic *et al*. 2016). It is expected that when rearing conditions change (e.g. host plant), organisms and populations adapt to the new conditions (Clarke 1998; Benítez *et al*. 2014a; Pieterse *et al*. 2017). Similar results have been reported using GM methods for other invasive species (e.g. *Diabrotica virgifera virgifera* LeConte, *Bothynoderes punctiventris* Germar), which have developed different biotypes under diverse environmental conditions (Lemic *et al*. 2014, 2016; Benítez *et al*. 2014a, 2014b). Knowledge of morphological differences in pests due to different rearing conditions in the host plant could facilitate monitoring of pest populations during their expansion into new areas, as well as in the development of the most effective agricultural protection measures (Lemic *et al*. 2016). Therefore, GM analyses could be an additional tool used to detect different biotypes of *D. suzukii* populations, as Schutze *et al*. (2012) did for the *Bactrocera* species complex. By GM screening of this insect species, different bioecological groups of *D. suzukii* could be detected, with the most invasive biotypes selected as a priority appropriate management methods applied rapidly. This method could be a supportive tool for recently developed geographic profiling as an efficient technique to track *D. suzukii* colonization patterns (Cini *et al*. 2012) and could help create standardized and area-wide sampling networks whose purpose is to improve the effectiveness of pest management decisions and prevent damage (Cini *et al*. 2012). Many different management techniques for *D. suzukii* are still under investigation (Cini *et al*. 2012), but the area-wide pest management techniques seem to be a crucial approach for effective control of these invaders.

The results of the present survey will serve as a starting point in further selection of different biotypes of *D. suzukii* at a wider geographic area, and could be linked with native pest populations.

Acknowledgments

This study was financed, in part, by a project grant from Chile and Croatia (DGI-UTA9719-17 to HAB). Work was financed by LIFE+ INSECTLIFE (LIFE13 ENV/HU/001092) project. The Dr Benitez was financed in part, by a Small Grant UTA 2017 9719-17.

References

Adams DC, Funk DJ (1997) Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: multivariate applications of the thin-plate spline. *Systematic Biology* **46**: 180–194. https://doi.org/10.1093/sysbio/46.1.180.

Adams DC, Rohlf FJ (2000) Ecological character displacement in Plethodon: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 4106–4111. https://doi.org/10.1073/pnas.97.8.4106.

Alibert P, Moureau B, Dommergues JL, David B (2001) Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. *Zoologica Scripta* **30**: 299–311. https://doi.org/10.1046/j.1463-6409.2001.00068.x.

Asplen MK, Anfora G, Biondi A *et al*. (2015) Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science* **88**: 469–494. https://doi.org/10.1007/s10340-015-0681-z.

Benítez H, Briones R, Jerez V (2008) Fluctuating asymmetry in two populations of *Ceroglossus chilensis* (Eschscholtz, 1829) (Coleoptera: Carabidae) in agroecosystem of *Pinus radiata* D. Don, Bio-Bio region. Chile. *Gayana* **72**: 131–139.

Benítez HA, Parra LE, Sepulveda E, Sanzana MJ (2011) Geometric perspectives of sexual dimorphism in the wing shape of Lepidoptera: the case of *Synneuria* sp. (Lepidoptera: Geometridae). *Journal of the Entomological Research Society* **13**: 53–60.

Benítez HA, Sanzana M-J, Jerez V, Parra LE, Hernandez CE, Canales-Aguirre CB (2013) Sexual shape and size dimorphism in carabid beetles of the genus *Ceroglossus*: is geometric body size similar between sexes due to sex ratio? *Zoological Science* **30**: 289–295. https://doi.org/10.2108/zsj.30.289.

Benítez HA, Püschel T, Lemic D, Čačija M, Kozina A, Bažok R (2014a) Ecomorphological variation of the wireworm cephalic capsule: studying the interaction of environment and geometric shape. *PLoS One* **9**: e102059. https://doi.org/10.1371/journal.pone.0102059.

Benítez HA, Lemic D, Bažok R, Gallardo-Araya CM, Mikac KM (2014b) Evolutionary directional asymmetry and shape variation in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae): an example using hind wings. *Biological Journal of the Linnean Society* **111**: 110–118. https://doi.org/10.1111/bij.12194.

Bjeliš M, Buljubašić I, Popović L, Masten Milek T (2015) Spread of the spotted wing *Drosophila* – *Drosophila suzukii* (Diptera, Drosophlidae) and new distribution records in Dalmatia region of Croatia. *OEPP/EPPO Bulletin* **45**: 214–217. https://doi.org/10.1111/epp.12205.

Bookstein FL (1991) *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, UK.

Calabria G, Maca J, Bachli G, Serra LPascual M (2012) First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. *Journal of Applied Entomology* **136**: 139–147. https://doi.org/10.1111/j.1439-0418.2010.01583.x.

Carreira VP, Soto IM, Fanara JJ, Hasson E (2008) A study of wing morphology and fluctuating asymmetry in interspecific hybrids between *Drosophila buzzatii* and *D. koepferae*. *Genetica* **133**: 1–11. https://doi.org/10.1007/s10709-007-9176-z.

Cini A, Ioriatti C, Anfora G (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology* **65**: 149–160.

Cini A, Anfora G, Escudero-Colomar LA *et al*. (2014) Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe. *Journal of Pest Science* **87**: 559–566. https://doi.org/10.1007/s10340-014-0617-z.

Clarke GM (1998) Developmental stability and fitness: the evidence is not quite so clear. *The American Naturalist* **152**: 762–766. https://doi.org/10.1086/286207.

Dudley R (2002) *The Biomechanics of Insect Flight: Form, Function, Evolution*. Press, Princeton, NY, Princeton University.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x.

Gidaszewski NA, Baylac M, Klingenberg CP (2009) Evolution of sexual dimorphism of wing shape in the *Drosophila melanogaster* subgroup. *BMC Evolutionary Biology* **9**: 110. https://doi.org/10.1186/1471-2148-9-110.

Gilchrist GW, Huey RB (2004) Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. *Integrative and Comparative Biology* **44**: 461–470. https://doi.org/10.1093/icb/44.6.461.

Grassi A, Palmieri L, Giongo L (2009) Nuovo fitofago per i piccoli frutti in Trentino. *Terra Trentina* **55**: 19–23.

Henderson A (2006) Traditional morphometrics in plant systematics and its role in palm systematics. *Botanical Journal of the Linnean Society* **151**: 103–111. https://doi.org/10.1111/j.1095-8339.2006.00526.x.

Hoffmann AA, Woods RE, Collins E, Wallin K, White A, McKenzie JA (2005) Wing shape versus asymmetry as an indicator of changing environmental conditions in insects. *Australian Journal of Entomology* **44**: 233–243. https://doi.org/10.1111/j.1440-6055.2005.00469.x.

Johansson F, Söderquist M, Bokma F (2009) Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. *Biological Journal of the Linnean Society* **97**: 362–372. https://doi.org/10.1111/j.1095-8312.2009.01211.x.

Jolliffe IT (2002) *Principal Component Analysis*, 2nd edn, Springer-Verlag, New York, NY.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**: 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x.

Lalouette L, Williams CM, Hervant F, Sinclair BJ, Renault D (2011) Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comparative Biochemistry* *and Physiology Part A: Molecular & Integrative Physiology* **158**: 229–234. https://doi.org/10.1016/j.cbpa.2010.11.007.

Lemic D, Benitez HA, Bažok R (2014) Intercontinental effect on sexual shape dimorphism and allometric relationships in the beetle pest *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). *Zoologischer Anzeiger* **253**: 203–206. https://doi.org/10.1016/j.jcz.2014.01.001.

Lemic D, Benítez HA, Püschel T, Virić Gašparić H, Šatvar M, Bažok R (2016) Ecological morphology of the sugar beet weevil Croatian populations: evaluating the role of environmental conditions on body shape. *Zoologischer Anzeiger* **260**: 25–32. https://doi.org/10.1016/j.jcz.2015.11.003.

Lunardi RR, Benítez HA, Câmara TP, Gomes LP, Arrais-Silva WW (2017) Head shape variation in response to diet in *Triatoma williami* (Hemiptera, Reduviidae: Triatominae), a possible Chagas disease vector of legal Amazônia. *Zoologischer Anzeiger* **267**: 187–193. https://doi.org/10.1016/j.jcz.2017.04.001.

Masten Milek T, Seljak G, Šimala M, Bjeliš M (2011) First record of *Drosophila suzukii* (Matsumara, 1931) (Diptera Drosophilidae) in Croatia. *Glasilo Biljne Zaštite* **11**: 377–382. [In Croatian].

Masten Milek T, Šimala M, Bjeliš M (2015) Spotted wing *Drosophila* (*Drosophila suzukii*) – fruit pest. *Glasilo Biljne Zaštite* **15**: 323–327. [In Croatian].

Mešić A, Pajač Živković I, Barić B, Duralija B (2017) Prve ekonomske štete od novog štetnika voća (*Drosophila suzukii*). *Zbornik sažetaka 12. znanstveno-stručnog savjetovanja hrvatskih voćara s međunarodnim sudjelovanjem: "Voćarstvo otočnog, obalnog i priobalnog područja* 37*"*. [In Croatian]

Mikac KM, Douglas J, Spencer JL (2013) Wing shape and size of the western corn rootworm (Coleoptera: Chrysomelidae) is related to sex and resistance to soybean maize crop rotation. *Journal of Economic Entomology* **106**: 1517–1524. https://doi.org/10.1603/EC13010.

Mikac KM, Lemic D, Bažok R, Benitez HA (2016) Wing shape changes: a morphological view of the *Diabrotica virgifera virgifera* European invasion. *Biological Invasions* **18**: 3401–3407. https://doi.org/10.1007/s10530-016-1252-9.

Monteiro LR (1999) Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Systematic Biology* **48**: 192–199. https://doi.org/10.1080/106351599260526.

OEPP/EPPO Diagnostics (2013) PM 7/115 (1): *Drosophila suzukii*. *OEPP/EPPO Bulletin* **43**: 417–424.

O'Higgins P (2000) The study of morphological variation in the hominid fossil record: biology, landmarks and geometry. *Journal of Anatomy* **197**: 103–120. https://doi.org/10.1046/j.1469-7580.2000.19710103.x.

Parsons SMA, Joern A (2014) Life history traits associated with body size covary along a latitudinal gradient in a generalist grasshopper. *Oecologia* **174**: 379–391. https://doi.org/10.1007/s00442-013-2785-6.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution* **25**: 459–467. https://doi.org/10.1016/j.tree.2010.05.006.

Pieterse W, Benítez HA, Addison P (2017) The use of geometric morphometric analysis to illustrate the shape change induced by different fruit hosts on the wing shape of *Bactrocera dorsalis* and *Ceratitis capitata* (Diptera: Tephritidae). *Zoologischer Anzeiger* **269**: 110–116. https://doi.org/10.1016/j.jcz.2017.08.004.

Polak M (2003) *Developmental Instability: Causes and Consequences*. Oxford University Press.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. *Trends in Ecology & Evolution* **8**: 129–132. https://doi.org/10.1016/0169-5347(93)90024-J.

Rohlf FJ, Slice D (1990) Extensions of the Procustes methods for the optimal superimposition of landmarks. *Systematic Zoology* **39**: 40–59. https://doi.org/10.2307/2992207.

Schutze MK, Jessup A, Clarke AR (2012) Wing shape as a potential discriminator of morphologically similar pest taxa within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae). *Bulletin of Entomological Research* **102**: 103–111. https://doi.org/10.1017/S0007485311000423.

Seljak G (2011) Spotted wing *Drosophila*, *Drosophila suzukii* (Matsumura), a new pest of berry-fruit in Slovenia. *Sadjarstvo* **22**: 3–5. [In Slovenian]

Siomava N, Wimmer EA, Posnien N (2017) Extensive sexual wing shape dimorphism in *Drosophila melanogaster*, *Ceratitis capitata*, and *Musca domestica*. *bioRxiv*. https://doi.org/10.1101/135749.

Soto I, Carreira V, Corio C, Soto E, Hasson E (2010) Host use and developmental instability in the cactophilic sibling species *Drosophila gouveai* and *D. antonietae*. *Entomologia Experimentalis et Applicata* **137**: 165–175. https://doi.org/10.1111/j.1570-7458.2010.01049.x.

Soto IM, Carreira VP, Soto EM, Hasson E (2008) Wing morphology and fluctuating asymmetry depend on the host plant in cactophilic *Drosophila*. *Journal of Evolutionary Biology* **21**: 598–609. https://doi.org/10.1111/j.1420-9101.2007.01474.x.

Upton MS, Mantel BL (2010) *Methods for collecting,s preserving and studying insects and other terrestrial arthropods*, 5th edn, CSIRO and the Australian Entomological Society, Camberra, ACT.

Valenzuela N, Adams DC, Bowden RM, Gauger AC (2004) Geometric morphometric sex estimation for hatchling turtles: a powerful alternative for detecting subtle sexual shape dimorphism. *Copeia* **4**: 735–742.

Wootton RJ (1981) Support and deformability in insect wings. *Journal of Zoology* **193**: 447–468.