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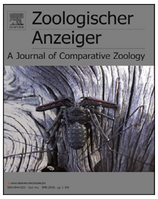

Research paper

Agroecological effect and sexual shape dimorphism in medfly *Ceratitis capitata* (Diptera: Tephritidae) an example in Croatian populations

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ABSTRACT

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann, 1824) is one of the most economically important fruit flies globally. Geometric morphometric methods based on principal component analyses, multivariate regressions and two blocks partial least squares analyses were used in this study to estimate patterns of sexual shape dimorphism and the influence of agroecological factors (weather conditions, soil properties) in two different populations on *C. capitata* in Croatia. Results shown significant differences in mean shape between *C. capitata* sexes, nevertheless, females did not modify they wing morphology between populations, in contrast to males where the wing shape differ between island and continent populations. Wing size and shape showed particularly differences between agro-ecological systems suggesting metapopulation structure of *C. capitata* which is currently favouring sex-specific plasticity. These results show that geometric morphometric procedures can be used to provide key insight into the biological adaptation of medfly in newly invaded habitats, a significant process that ensures invasion success.

1. Introduction

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann, 1824) is an invasive tephritid species originating from eastern Africa (De Meyer et al. 2002). The medfly firstly invaded the Mediterranean basin in second half of 19th century (Papadopoulos 2008) and then colonized tropical and mild temperate habitats around the world (Gilstrap & Hart 1987). Today it inhabits five continents (De Meyer et al. 2008) and is one of the world's most destructive pests of horticulture (Liquido et al. 1991; Vera et al. 2002). It is extremely polyphagous, with more than 350 different reported hosts and attacks the ripening fruit of numerous commercially important fruit crops (Liquido et al. 1991). *C. capitata* is a multivoltine species and its development depends on the geographic area, climatic conditions, and availability of host plants (Bjeliš et al. 2014). The movement of *C. capitata* is usually assisted as it occurs through the small and large scale movement of commodities through international and national trade routes (White & Elson-Harris 1994; Ordax et al. 2015). Many aspects of the population dynamics of *C. capitata* have been studied during the last few decades. That is, *C. capitata* require food, mates, egg-laying sites, and refugia as essential resources (Prokopy et al. 1994). In many studies the temporal patterns of adult captures and habitat preferences of *C. capitata* have been investigated (Papadopoulos et al. 1996; Katsoyannos et al. 1998; Papadopoulos et al. 2001). The importance of the abundance and distribution of host trees on the spatial dispersion patterns of *C. capitata* has been proposed in the past (Papadopoulos et al. 1996; Katsoyannos et al. 1998; Papadopoulos et al. 2001) including how aggregate in response to a changing environment depending on sex (Papadopoulos et al. 2003).

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Meats & Smallridge (2007) have shown that *C. capitata* populations exhibits short- and long-range dispersal, concluding that this could facilitate its invasiveness. Larger sized *C. capitata* are capable of long-distance migration and can move a distance of at least 50 km (Israely et al. 2005). Navarro-Campos et al. (2011) investigated the influence of host fruit and temperature during larval development on adult body size (e. g. wing area) in the laboratory and versus field conditions. These authors found that the size of flies collected in the field varied seasonally; the largest individuals were found during the winter–spring period while the smallest were collected during summer. Overall, the size of flies was more related to air temperature than with fruit host (Navarro-Campos et al. 2011). Fitness and morphometric traits (e. g. eye length, head width, thorax length, face width and wing length) of laboratory reared and wild males in breeding with wild females was investigated by Rodriguero et al. (2002). Overall, the authors found that wild medfly were on average bigger than laboratory reared individuals. Nevertheless, all the morphometric traits analysed correspond to morphological variation in size (Rodriguero et al. 2002; Navarro-Campos et al. 2011). Research of geometric morphometrics (GM) tools that consider the combination of wing shape and size for *C. capitata* research are still understudied.

Geometric morphometrics can provide valuable information on phenotypic variability and population structure with the advantage of being a low-cost and rapid tool (Adams et al. 2013; Klingenberg 2013; Benítez and Püschel 2014). Geometric morphometric techniques assess shape changes and can uncover uneven components of shape change (Thin Plate Spline); this shape change then represents all the movements of landmarks and can define changes occurring in specific points of the shape. Using geometric morphometric tools, the size and shape of the wing can be analysed independently to untangle biological patterns related to heritability and environmental sensitivity (Klingenberg 2010).

Despite its proven utility for insect wing analysis (Mikac et al. 2013, 2016, 2019; Benítez et al. 2014a, 2014b; Lemic et al. 2014; Pajač Živković et al. 2018, 2019) there have been very few studies of *C. capitata*. The use of geometric morphometric analysis has been recently applied to *C. capitata* to understand mating success and associated wing shape changes in sterile and wild males (De Souza et al. 2015). Pieterse et al. (2017) later investigated the influence of nutritional stress (e. g. different fruit hosts) on *C. capitata* wing shape.

Given its cosmopolitan distribution, invasive nature and its abundance and ability to thrive in numerous environments, *C. capitata* is an ideal model species to investigate the effects of environmental factors on size and shape variation.

In this study we investigated the relationship between morphological variation in *C. capitata* populations in different agroecological systems (climatic and edaphic different agroecosystems) in Croatia. We hypothesized that Croatian *C. capitata* populations vary at the geographical and agroecological level and that environmental variables (temperature, rainfall, humidity or soil properties) affect wing size and shape. To test this hypothesis, the wing structure of *C. capitata* was evaluated using morphometric traits, and the environmental effect on wing size and shape determined. We expected to detect contrasting patterns of morphological variation in the wings – a structure mostly related to invasion biology of this insect pest.

2. Materials and methods

2.1. Data collection

In autumn 2019 mandarin fruit with symptoms of *C. capitata* infestation were collected from two different locations in Croatia. First location was Island of Brač (BRA), and the second, costal location was Dubrovnik (DUB). The distance between Dubrovnik and Brač is approximately 180 km and requires ferry transportation. The fruit were transported to Department for Agricultural Zoology, Faculty of Agriculture Zagreb and stored in separated entomological cages in temperature-controlled cabinets at 25 °C and a relative humidity of 75%. Fully developed adults were collected after a two weeks period and stored in 70% ethanol pending further analyses. Species and sex were determined through the examination of the abdominal apex prior to wing dissection (White & Elson-Harris 1994). The left and right wing of each fly was removed and side-mounted using the fixing agent Euparal (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) based on standard methods (Upton & Mantel 2010) for subsequent morphometric analysis (Fig. S1). In total 53 (30 females, 23 males) *C. capitata* from Brač island and 51 (25 females, 26 males) *C. capitata* from costal area of Dubrovnik were analysed via the methods described hereinafter.

2.2. Environmental factors

Climate data used in this study (i.e. mean air temperature, mean relative humidity and total rainfall) were obtained from the Croatian Meteorological and Hydrological Service. The distance between the meteorological stations and trapping localities was a maximum distance of 20 km.

From all locations were mandarin fruit were collected, soil samples were collected from the depth of a plow layer (30 cm). In each field, five sub-samples (each 300–400 g in weight) were taken, sub-sampling sites were spaced 30–40 m apart depending on size of field sites. The five sub-samples were then pooled and homogenized and a sub-set of the pooled soil from each site was analysed. Sediment grain size and chemical property analyses were conducted at the pedology laboratory of the Department of Soil Science, Faculty of Agriculture, University of Zagreb. The following soil parameters were investigated: percentage coarse and fine sand, coarse and fine silt, and clay, humus and pH in H_2O and KCl. Soil texture was determined by sieving following standard methods (ISO 11277 2004). Soil humus (0.3000 g sample weight) was determined by a volumetric titrimetric wet combustion method. Differences in environmental properties of both locations were tested using ANOVA in JMP vPro 14.

2.3. Multivariate analysis of shape

Geometric morphometric analyses were performed using images of the left and right wings of *C. capitata* individuals both wings were used as matched symmetry, that mean they are present in two separate copies on both body sides that are mirror images of each other. This type of symmetry is called matching symmetry because the left and right copies can be matched to each other after one of them has been reflected to its mirror image. The wings pictures were taken by a Leica DFC295 digital camera (3 M Pixel) on a trinocular mount of a Leica MZ16a stereo-microscope and saved in JPEG format using the Leica Application Suite v3.8.0 (Leica Microsystems Limited, Switzerland).

Fourteen landmarks (LMs: anatomical homologous points) were digitized on every image using the software tpsDig v2.10 (Rohlf 2008) (Fig. 1). X–Y coordinates were obtained for all landmarks and the shape information was extracted using a Procrustes superimposition procedure (Rohlf & Slice 1990; Dryden & Mardia 1998). This procedure removes the information of size, position and orientation to standardize each specimen according to centroid size. The shape variation between sexes and populations were analysed using principal components analysis (PCA). To evaluate

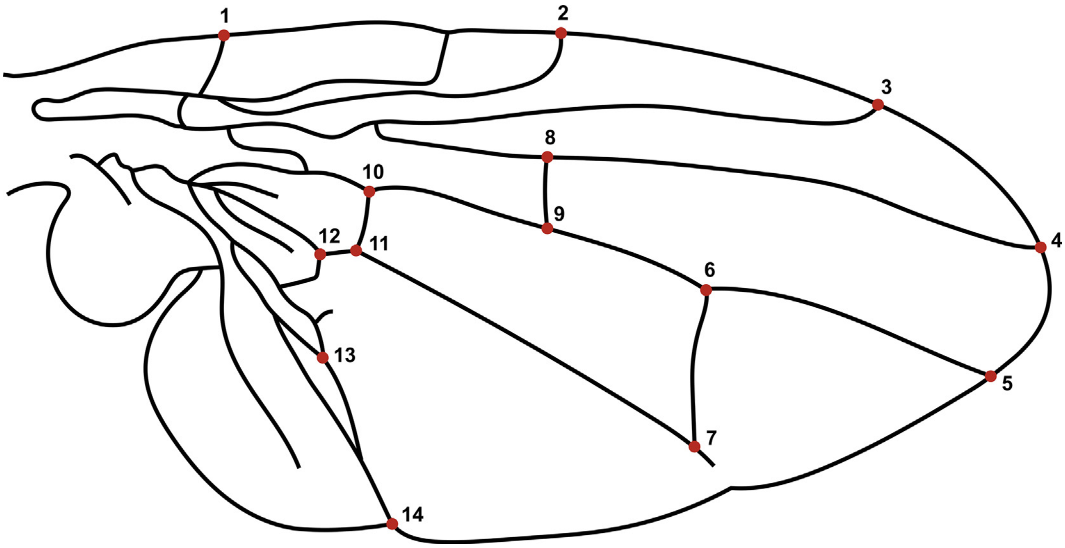

Fig. 1. Graphical representation of fourteen landmarks in *Ceratitis capitata* wing.

the influence of static allometry in the data, a multivariate regression of shape as a dependent variable (Procrustes coordinates), on centroid size (independent variable) was calculated. Further, to investigate the influence of environmental conditions between island and coastal geography on wing shape, a two-block partial least squares analysis (PLS) (Rohlf & Corti 2000) was performed, using the 13 different environmental variables previously stated for each sampling location. This analysis is based on the singular value decomposition of the common covariance matrix of the two variables sets, which in our case consist of the shape and climatic/soil conditions matrices. The significance of the correlation between the pairs of singular vectors (i.e., climatic/soil variable singular vectors and shape singular vectors), was tested (permutation test: 10,000 randomizations) (Benítez et al. 2014; Lemic et al. 2016). Finally, the differences between groups, a mixed classifier was made between population (island vs. coast) and sex and a canonical variate analysis (CVA), was performed to find the shape characters that best distinguish among groups of specimens. These analyses were undertaken in MorphoJ v1.05d (Klingenberg 2011).

3. Results

Climate data (mean air temperature, mean relative humidity and total rainfall) were analysed per field site (Fig. 2). The physicochemical soil properties are presented in Table 1. Significant differences in climate conditions based on geography were found (temperatures: $P < 0.001$; precipitation: $P < 0.001$; soil: $P < 0.05$); though no significant difference was revealed for humidity data ($P = 0.785$).

Morphological variability was visualized by the scatterplot of the PCA (Fig. 3). The first three PCs accounted for 41.1%, 12.4% and 7.3% of the total shape variance, respectively. In PC1 patterns related to sexual shape dimorphism were found, where sexes are graphically separated (negative values had all males and positive values associated with all females), this pattern was represented for both populations studied (Fig. 3A).

After averaging data, the Averaged PCA showed a similar structure of the data with similar wings between females' populations and some disparity between the average shape of male populations, the shape variation of the extreme values of the PCA shows particularly variation related to the wing widening (Fig. 3B). Due to the normal PC1 variation is related to the allometry component, our results showed minimal size influence between males and females, where the multivariate regression revealed no allometry in the wing shape (>5% of allometric influence) (Fig. 4). After superimposing the average shape of every population and sex in one image, shape variation was noticeable in females via the elongation of the terminal landmarks 3, 4 and 5 of the veins. For males, a broadening of the wing was more evident by the expansion of the landmarks 1,2 and 14. Independently of the apparent sexual shape dimorphism, the population differentiation between island and coastal locations was not well defined for shape (Fig. 5).

The two-block, partial least-squares analyses of the wing shape and environmental covariates were slightly more informative for the population analysed, showing a lower association between the shape variables and the environmental conditions. The RV coefficient was static as the same environmental condition was tested for every specimen ($RV = 0.0563$: with a correlation of $r = 0.305$). Nevertheless, the significance of the relationship was different depending the tested variables (temperature: $p = 0.0374$; precipitation: $p = 0.0425$; humidity: $p = 0.0419$; soil traits: $p = 0.0407$). Finally, the CVA of the combined classifier showed that the new CV axes were able to distinguish the males of continent and island population, nevertheless, females remained undifferentiated (Fig. 6).

4. Discussion

This study found: (i) an overall wing size and shape similarity among *C. capitata* populations; (ii) a non-significant effect of environmental factors on wing shape; and (iii) a moderated male population differentiation using wing shape data.

Centroid size was used as a measure of overall wing size differences among *C. capitata* populations. The slight variation in wing size among populations suggests that either this trait is not a target of strong differential selection or that wing size in *C. capitata* is well suited to changing environmental conditions (Papadopoulos et al. 1996, 2001; 2003; Katsoyannos et al. 1998; Navarro-Campos et al. 2011). In contrast to size, analyses of organismal shape can provide more reliable information about phenotypic variation of populations representing high and stable heritability (Moraes et al. 2004; Dujardin 2008).

Although mean wing shape comparisons revealed significant differences between populations, clear site-specific population

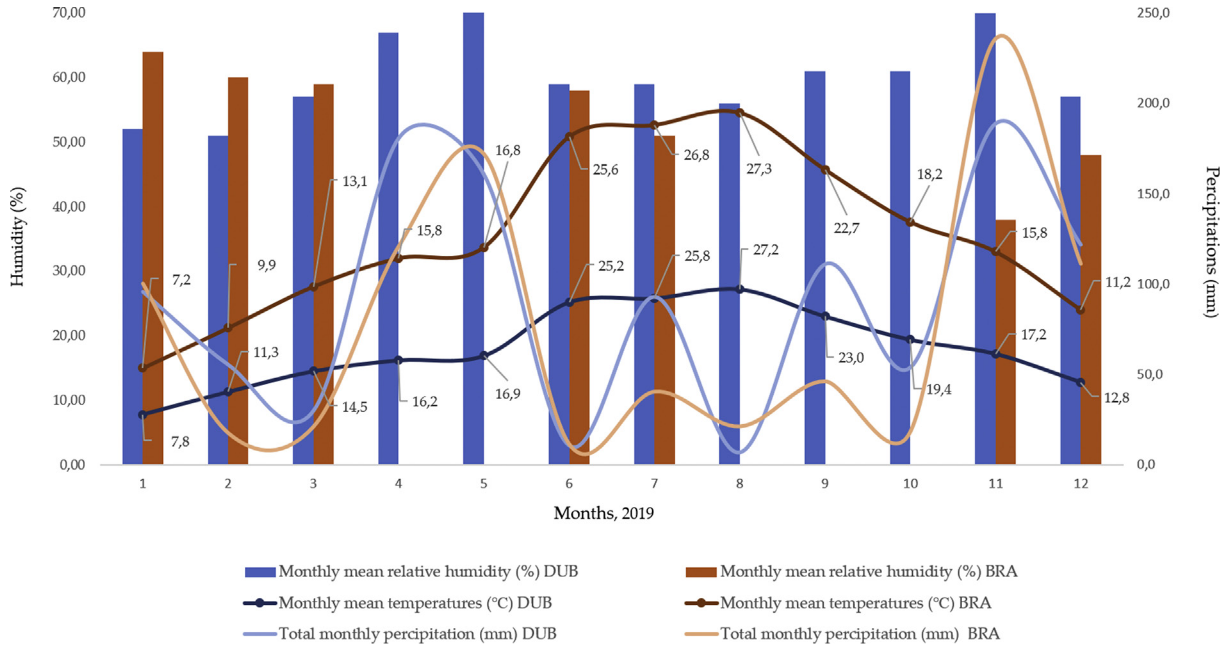

Fig. 2. Climate data (mean air temperature, mean relative humidity and total rainfall) for locations Brač (red) and Dubrovnik (blue) analysed per month. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Physical and chemical soil properties on both locations.

Soil properties	Brač Island	Dubrovnik
Coarse sand (%)	5.20	1.50
Fine sand (%)	11.90	4.50
Coarse silt (%)	18.10	13.30
Fine silt (%)	30.20	54.20
Clay (%)	34.60	26.50
Soil pH in H_2O	7.44	7.62
Soil pH in KCl	7.01	7.30
Humus (%)	6.96	3.72

differentiation was not found (see Fig. 5). However, an agroecological pattern in wing shape variation was detected when populations were pooled by sex. Both populations investigated in this study showed high levels of sex based shape dimorphism. Sex based dimorphism is often revealed in many species of Diptera, Lepidoptera, Hymenoptera and Coleoptera (Bonduriansky 2006; Gidaszewski et al. 2009; Marsteller et al. 2009; Benítez et al. 2011, 2013; Lemic et al. 2014). For *C. capitata* this sexual dimorphism was most important in landmarks 1, 2, 3, 4, 5, and 14. These landmarks relate to the radial veins, medial and anal vein and are a key anatomical character used to distinguish different wing morphotypes in tephritids (Van Cann et al. 2015). Both for island and costal populations in this study, female *C. capitata* had more elongated

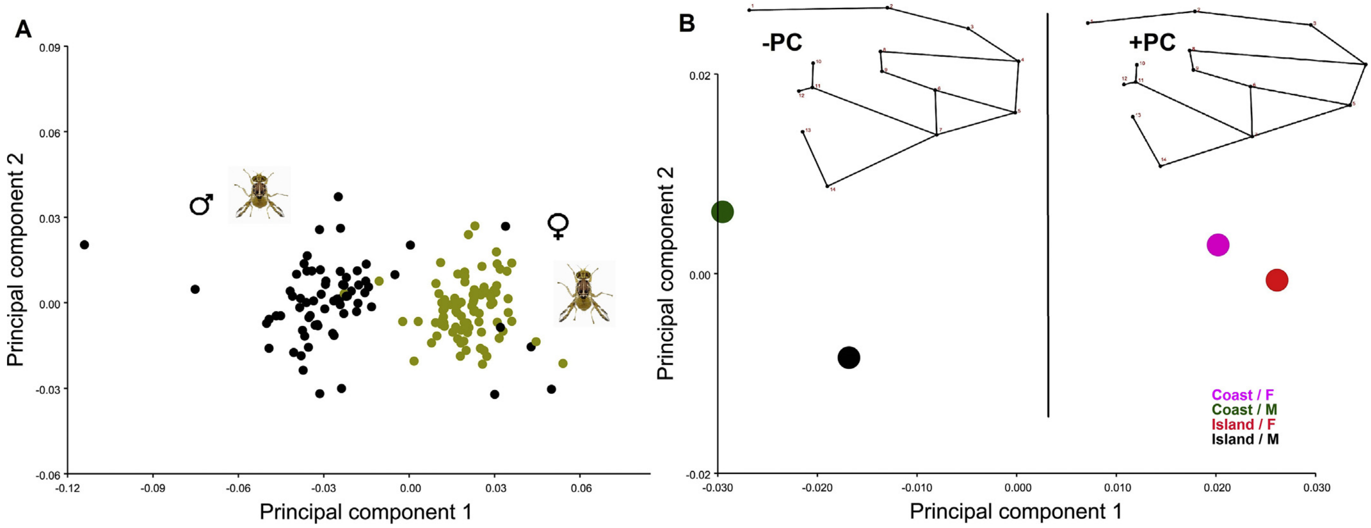

Fig. 3. Principal component analysis of wing shape variation in *Ceratitis capitata*. A: Representation of the sexual shape dimorphism, Dark Yellow: Females Black: Males, B: Average PCA between population and sex with the extreme negative and positive wing shape wireframe, Pink: Coastal female, Green Coastal male, Red Island female and Black Island male. Each point represents a different wing shape. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

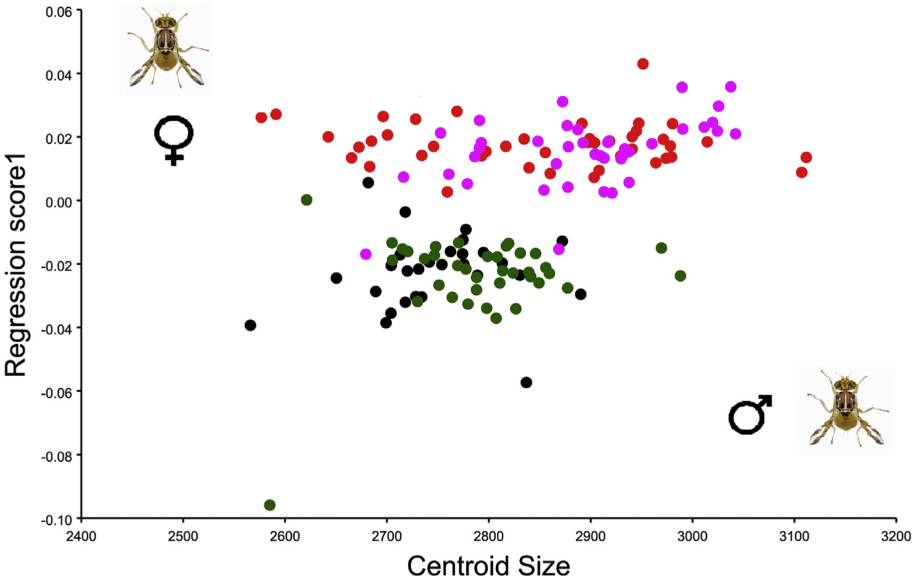

Fig. 4. Multivariate regression analysis of shape as a dependent variable (y-axis Regression scores 1) and size (x-axis centroid size) as an independent variable of *Ceratitis capitata*. Pink: Coastal female, Green Coastal male, Red Island female and Black Island male. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

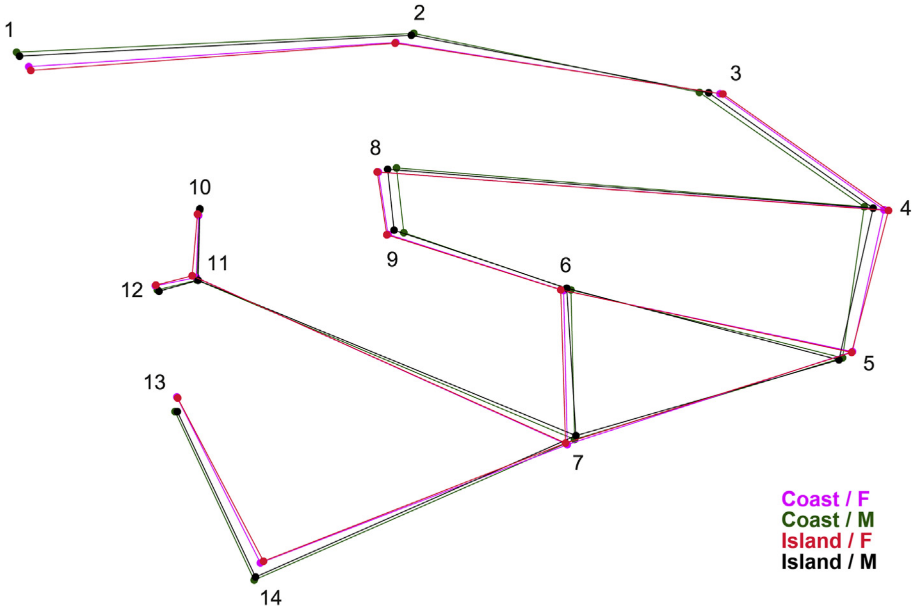

Fig. 5. Wireframe representation of the superimposition between the average wing shape between male and females, and their corresponding island and coastal populations.

wings than males. Since elongated wings are considered to be found in the sex more involved in migratory movements (i.e. milkweed bugs: Dingle 1981; monarch butterflies: Dudley & Srygley 1994; Altizer & Davis 2010), this study provides morphological evidence that most migration in *C. capitata* can be attributed to the females of this species. Also, as discussed in Mikac et al. (2013) for coleopteran species it is thought that elongated wings are probably more aerodynamic and may also be useful for mated females that are known to engage in migratory flights over relatively long distances. In contrast, male *C. capitata* have shorter and wider wings (Churchill-Stanland et al. 1986). It is known that male *C. capitata* establish mating territories and attract females for mating and as such there is little reason for males to undertake long distance flights to find females (Hendrichs et al. 2002). It is most likely that the sexual dimorphism in wing shape of *C. capitata* may be explained by sex-specific dispersal requirements of this species.

At the population level, the variation of the first two principal component showed that there was no clear-cut separation between the populations, which is in agreement with a high proportion of misassignments and the findings of low population structure (using biochemical and molecular markers), as demonstrated by Gasparich et al. (1997) and Gasperi et al. (2002) who revealed isolation by distance in Mediterranean populations. According to these authors findings' *C. capitata* populations are genetically similar throughout the Mediterranean. The slight wing shape differences detected (especially between sexes) may be the result of

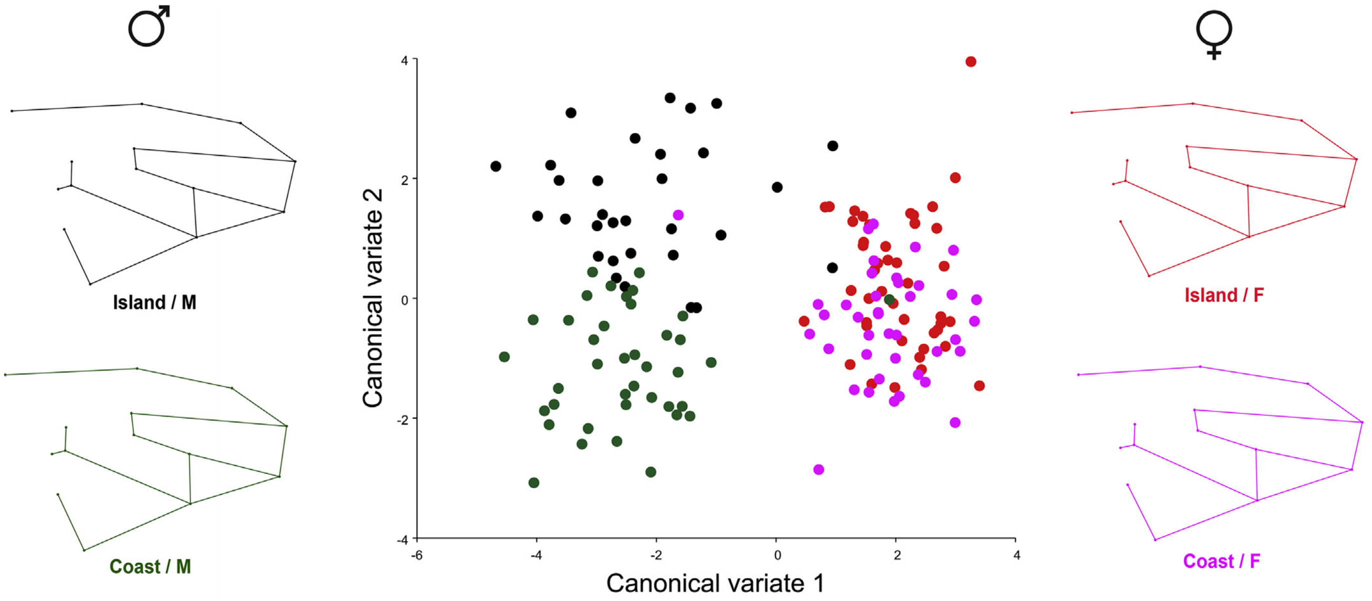

Fig. 6. Canonical variate analysis showing the discrimination in wing shape between populations and sex as a combined classifier for *Ceratitis capitata*.

emerging phenotypic plasticity. Phenotypic plasticity is often defined as the change in phenotypic expression of a genotype in response to environmental factors (Schlichting 2004) and has been shown to have significant evolutionary consequences (Schlichting 2004; Murren et al. 2005). Many studies have suggested that newly invasive species have higher levels of phenotypic plasticity, but empirical tests of this theory are less common (Hulme 2008; Davidson et al. 2011). While it is considered to be invasive species, *C. capitata* has been present in Croatian littoral regions for over fifty years (Kovačević 1960). *C. capitata* in this period has become well established in the Dalmatian region attacking numerous fruit hosts (apricot, peach, nectarine, plum, fig, apple, pear, mandarin, orange and persimmon). Beside cultivated crops, *C. capitata* has been confirmed to attack also numerous ornamental and wild host plants like strawberry tree, loquat, kumquat, feijoa and sour orange (Bjeliš et al. 2014). *C. capitata* establishment of more than fifty years and numerous host plants for this invasive species has likely resulted in low need for phenotypic plasticity and total adjustment to agroecological and environmental factors there. Low morphological variability between population from clearly very different agroecological systems investigated in our study, indicate stability in the *C. capitata* genotype which is reflected by a stable phenotype (see Bouyer et al. 2007). We show metapopulation structure of *C. capitata* in differing Croatian agroecological systems which is currently favouring sex-specific plasticity. This can be a relevant characteristic of invasive species living under variable environmental conditions that are colonizing new habitats.

Significant differences in mean shape between *C. capitata* sexes were detected, but they were smaller than those at the intra-population level (sexual dimorphism). Wing size and shape were similar among populations with no significant differences between agroecological systems. Overall, site-specific population differentiation was not strongly supported by wing traits. The lack of a significant relationship among geographic, environmental and phenetic distances suggests that geographical distance cannot explain the slight wing shape differentiation pattern found among *C. capitata* populations studied here. It is possible that the short flight range of *C. capitata* and similar environmental conditions among some localities favour wing shape similarity. Both datasets consistently reinforced a panmictic population of *C. capitata* across the locations investigated in Croatia.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2020.08.005.

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