

Article

Medfly Phenotypic Plasticity as A Prerequisite for Invasiveness and Adaptation

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Abstract: The hypothesis of this study was that different plant hosts of the medfly *Ceratitis capitata* may cause variability as a prerequisite for its invasiveness. The main objective was to determine population variability based on medfly wing shape in three favorable medfly host plants (peach, fig and mandarin) from different agroecological growing areas with different pest management practices, and to evaluate phenotypic plasticity as a basis for future expansion into new areas and new hosts. Using geometric morphometric methods across 14 specific landmarks on the medfly wings, 10 populations were tested from infested peach, fig and mandarin fruits, as well as laboratory-grown sterile populations. The studies led to the following main findings: (1) all of the medfly populations that were studied exhibited sexual dimorphism in wing shape; (2) the hosts in which the medfly develops influence wing shape and condition its variability; (3) there is significant variability between laboratory mass-reared sterile and wild individuals in male and female populations; (4) a high phenotypic plasticity of medfly populations was observed along the study sites. Even the low but clearly detected variability between different agroecological conditions and localized variability indicate genotypic stability and high phenotypic plasticity, which can be considered as a prerequisite for medfly invasiveness and dispersal to new areas.

Keywords: *Ceratitis capitata* (Wiedemann); geometric morphometrics; invasiveness; spreading potential; plasticity



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1. Introduction

The Mediterranean fruit fly or medfly (*Ceratitis capitata* (Wiedemann 1824)) is considered to be one of the most important tephritid fruit fly species worldwide and is an extremely dangerous polyphagous pest of fruits and vegetables that has been reported in five different continents in temperate, subtropical and tropical climates [1]. The medfly originated in tropical sub-Saharan Africa [2], from where it was introduced to the Iberian Peninsula in 1842 [3] and subsequently spread to Italy in 1863 and to France in 1885 [4], and further to the temperate areas of Mediterranean countries [5]. Further spread into the interior of Europe [3,6–10] was due to the intensive international trade in infested fresh fruits of various host plants, which is considered to be the main cause of the invasion of the pest [11–13]. In temperate areas of the Mediterranean, such as northern Greece and the Croatian coastal area, the medfly completes four to five generations per year due to its high biotic potential and adaptability to different ecological conditions [6,14,15], the duration of its life cycle being determined by temperature conditions and the nutritional value of the

host fruits on which the larvae feed [16]. Its specific adaptive biology, invasive character and climate change have a very favorable effect on the establishment of its populations in new areas [3,17–19]. Today, *C. capitata* is distributed on five continents and in over 120 countries [20]. It is the most important invasive tephritid fruit fly species in fruit crops worldwide [21–23], infesting over 260 different plant species [3,24,25].

Recent reports and examples of the spread and establishment of the medfly pose a threat to inland Croatia, as well as to other fruit growing areas in surrounding European countries, which could seriously threaten local fruit production and the fruit industry [26–30]. High phenotypic plasticity is one of the main characteristics of invasive organisms. Phenotypic plasticity is defined as a change in the expression of the phenotype due to a change in the genotype under the influence of various environmental factors [31,32]. This phenomenon has been shown to have serious implications for evolution [33,34]. The high plasticity of the organism is the cause of the formation of different phenotypes that facilitate populations to resist changing environmental factors and to establish and maintain a population in a given area [35–37]. The extent of phenotypic plasticity is one of the main differences between invasive and non-invasive species, especially in animals [38]. The study of the invasiveness, variability and dispersal pathways of pests is extremely important for the appropriate application of control measures. In addition to the use of expensive genetic markers, changes in the variability of different populations of organisms can be studied using geometric morphometric methods [39–41]. Indeed, Camara et al. [42] and Bouyer et al. [43] show that genetic markers (such as microsatellites) reveal major changes in the genome of a given population, while it is more effective to use morphometric markers to identify minor (and recent) genetic changes. Thus, this is a “type” of biomarker that can be used to successfully detect and describe the changes in phenotype that occur under the influence of genotype.

Geometric morphometrics (GM) is a combination of morphological measurements and multivariate statistical methods used to process these data. It is based on the placement of certain points (markers) on certain parts of the body; in insects these are usually the wings, as they are responsible for the rapid dispersal of insects (fliers) to new areas. Certain points are placed in a specific order at the intersections of the wings and form two-dimensional shapes in the coordinate system, which are then evaluated using various statistical methods [44]. In the last two decades, GM has been used very successfully in the study of the genetic variability of various organisms, especially in insects of the order Diptera [45–51]. Lemic et al. [52] studied the influence of the various agroecological conditions of mandarin cultivation on the wing shape of the medfly and demonstrated that similar environmental conditions favor wing shape similarity, which reinforced the panmictic population in a specific geographic area. This can be a relevant characteristic of invasive species living under variable environmental conditions that are colonizing new habitats [52]. The specific biology and behavioral complexity of the medfly indicate the extremely invasive nature of this pest. The invasive nature of these organisms is the result of the great ability of the organism to adapt quickly to different ecological conditions.

Based on the above, this article hypothesized that the different plant hosts in which the medfly has evolved are the cause of the variability of the pest as a prerequisite for its invasiveness. The objectives were: (i) to determine the variability of populations based on the wing shape of the medfly developing in the fruits of different hosts (peach *Prunus persica* L., fig *Ficus carica* L. and mandarin *Citrus reticulata* B.) in different cultivation areas in the Central Dalmatia and Southern Dalmatia region of the Republic of Croatia; (ii) to determine the variability of wing shape between different wild populations of medfly and laboratory-bred sterile medflies, and to evaluate the possible impact of morphological traits on the competitiveness of sterile males and the effectiveness of the sterile insect technique (SIT) in controlling this pest; and (iii) determine the phenotypic plasticity of the medfly and evaluate the potential for the spread of this invasive species into new areas and new hosts.

2. Materials and Methods

2.1. Data Collection

Adult fly populations were developed from fruit that was infested in the field from three different host plants: peach, fig and mandarin from six locations (see details in Figure 1 and Table 1) with different agroecological conditions and different pest management practices. Laboratory-bred sterile flies were also used for the study.



Figure 1. Location map.

Table 1. Overview of the origin of populations: Location, host, number of individuals and time of adult emergence from field infested hosts fruits.

Location	Plant Host	Control Measures *	Total Number of Individuals	Adult Emergence from Field Collected Fruits
Sućuraj	Fig	CON	40	10.10.–26.10.2020.
Hvar	Fig	CON	40	10.10.–26.10.2020.
Hvar	Mandarin	CON	40	20.10.–30.10.2019.
Podstrana	Fig	CON	36	10.10.–28.10.2020.
Podstrana	Peach	CON	39	1.09.–7.09.2020.
Metković	Mandarin	IPM	40	1.11.–14.11.2019.
Opuzen	Peach	SIT	40	13.07.–27.07.2020.
Opuzen	Fig	SIT	31	27.08.–8.09.2020.
Ston	Mandarin	CON	40	10.–28.11.2019.
Laboratory	Sterile	-	40	2019.

* IPM: integrated pest management; SIT: sterile insect technique; CON: conventional pest management.

During the summer and fall of 2019 and 2020, samples of peach, fig and mandarin fruit with visible symptoms of medfly infestation were collected from commercial orchards. The infested fruits were placed in the laboratory chambers. The conditions in the chambers were controlled (humidity 70% and temperature 25 °C). After their emergence at 2–5 days of age, the adult flies were collected and stored in 70% ethanol. The laboratory-bred sterile flies, strain Vienna 7, of both sexes, were obtained from Biofly, Sde Eliyahu, Israel. Before dissection of the wings [53], the flies were separated by sex according to male and female morphological characteristics by using binoculars. The left and right wings of each fly were

removed and laterally fixed using Euparal fixative (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) according to the standard methods [54] for subsequent morphometric analysis. From each population, 30 to 40 individuals were included in the study (care was taken to maintain an equal sex ratio).

2.2. Data Curation and Geometric Morphometric Analyses

In this study, a total of 386 individuals (772 wings) of medfly were analyzed. After dissection, the wings were photographed with a binocular loupe and camera and saved in JPEG format. On each photo of the medfly wing, 14 landmarks were placed at the nodes or ends of the veins. The landmarks were placed on the wings according to a well-defined arrangement (according to Bookstein [55] and published in Lemic et al. [52]) in the software package tpsDig V2.10 [56].

To analyze the coordinates of the landmarks, shape information was extracted with a Procrustes fit procedure using MorphoJ 1.06d software [57]. This procedure removes the size, position, and orientation information in order to standardize each specimen based on centroid size [58]. To distinguish between sexes and populations, a principal component analysis (PCA) was performed using the covariance matrix of the individuals' wing shape. A multivariate regression was performed to analyze whether there was an effect of size on wing shape (Allometry). Finally, to highlight the differences between the host and population groups, a Canonical variate analysis was performed as a discriminant analysis, where the axes of the PCA were modified using the maximum variation in order to create new axes and order the individuals according to the selected groups.

3. Results

Variability in wing shape for all of the populations that were studied was determined by principal component analysis (PCA) and shown in Figure 2A. PCA is performed to determine the overall variability among the studied populations. It was found that the first three principal components accounted for 70% of the total variability; PC1 explained 56%, and PC2 and PC3 explained 13% of the variability that was found. Analysis of the principal components of PCA revealed variability in wing shape between sexes, which was defined as sexual shape dimorphism. This was found for all of the populations that were studied.

Figure 2B shows a multivariate regression where the independent variable is the size of the wing (using the centroid size), and the dependent variable is the shape. The graph shows that there is no effect of size, suggesting that wing shape dimorphism is due only to influences of shape.

Extreme shape differences were graphically represented using a frame (configuration defined by 14 landmarks describing the shape of the veins) (Figure 3). After superimposition of the obtained average wing shape for each population and sex separately, some variability was observed. The variability in females was manifested as elongation of the terminal veins or changes in the positions of specific landmarks 3, 4, 5, 8 and 9, while in males, wider wings were observed due to changes in the positions of landmarks 1 and 2, and with more variance in landmark 14. Nevertheless, a clear difference can be observed when sexual dimorphism is evaluated in different populations where females tend to have more intra-variability than males from landmarks 11 and 14. This means that females have longer and stronger wings, which are characteristic of long flights, while males have narrow and short wings, which are characteristic of individuals that do not migrate much.

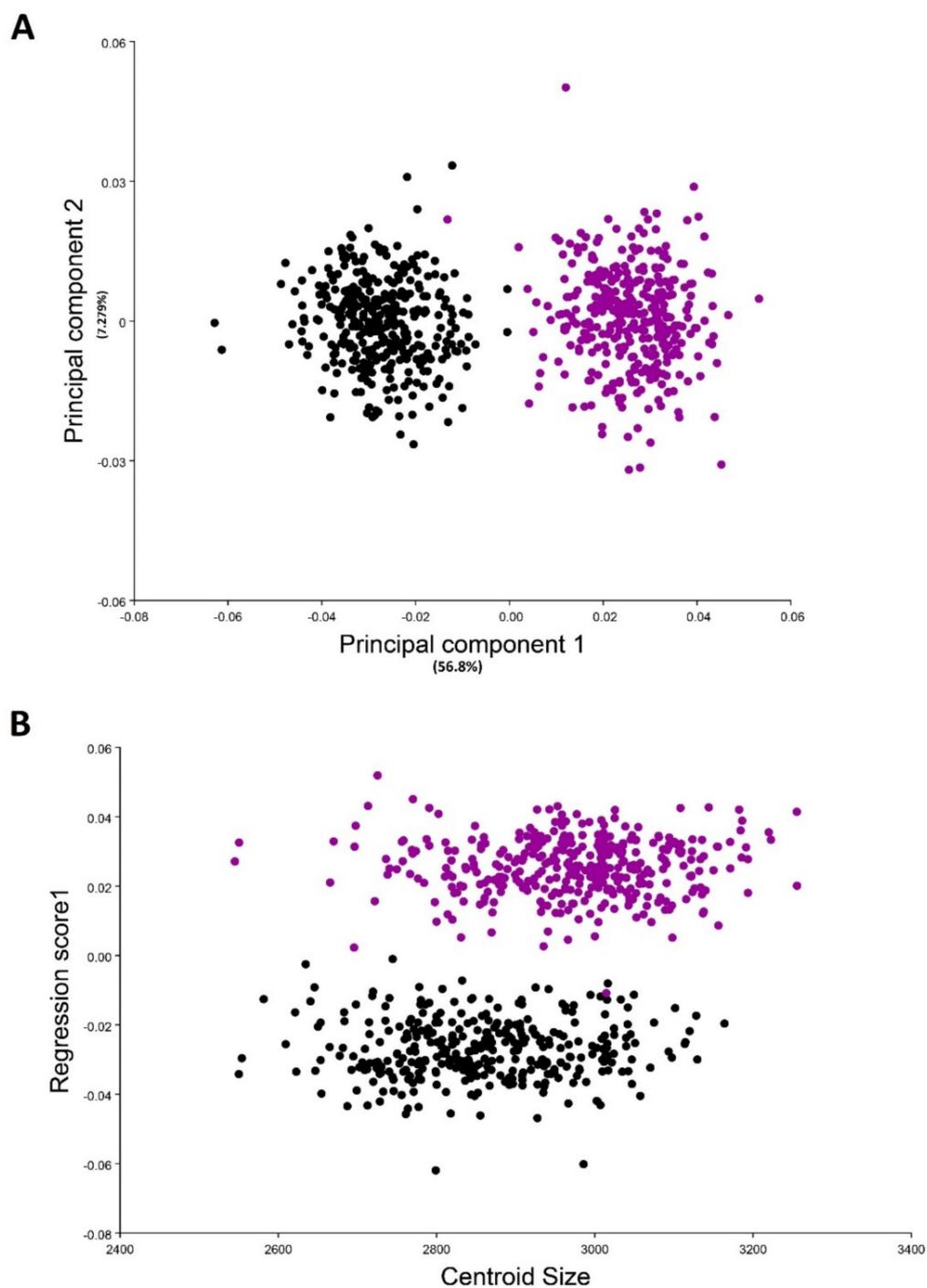


Figure 2. Sexual shape dimorphism of the medfly (black: males; purple: females) Analyzed by (A) Principal Component analyses and (B) Multivariate Regression of Shape vs. Centroid size.

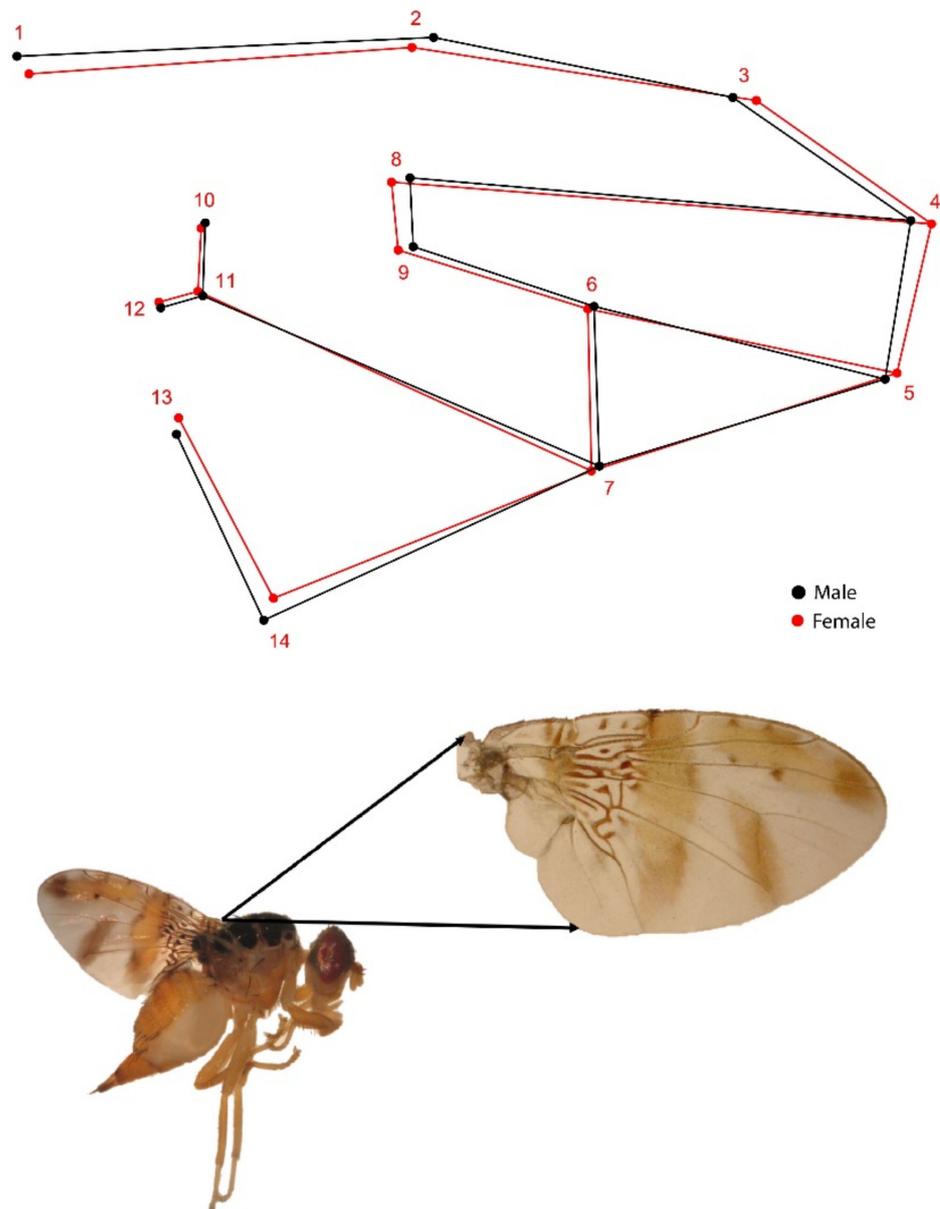


Figure 3. Wireframe of the wings of the medfly. The black lines show the schematic shape of the wings of males, the red wings of females.

3.1. Variability in the Shape of the Wings of the Medfly from Different Fruit Hosts

A total of 392 female and 380 male medflies were analyzed by CVA. For females, it was found that 45% of the variability could be explained by the first two axes (CV1 and CV2); similarly, 41% of the variability of males was explained by the first two canonical axes (CV1 and CV2) (Figure 4).

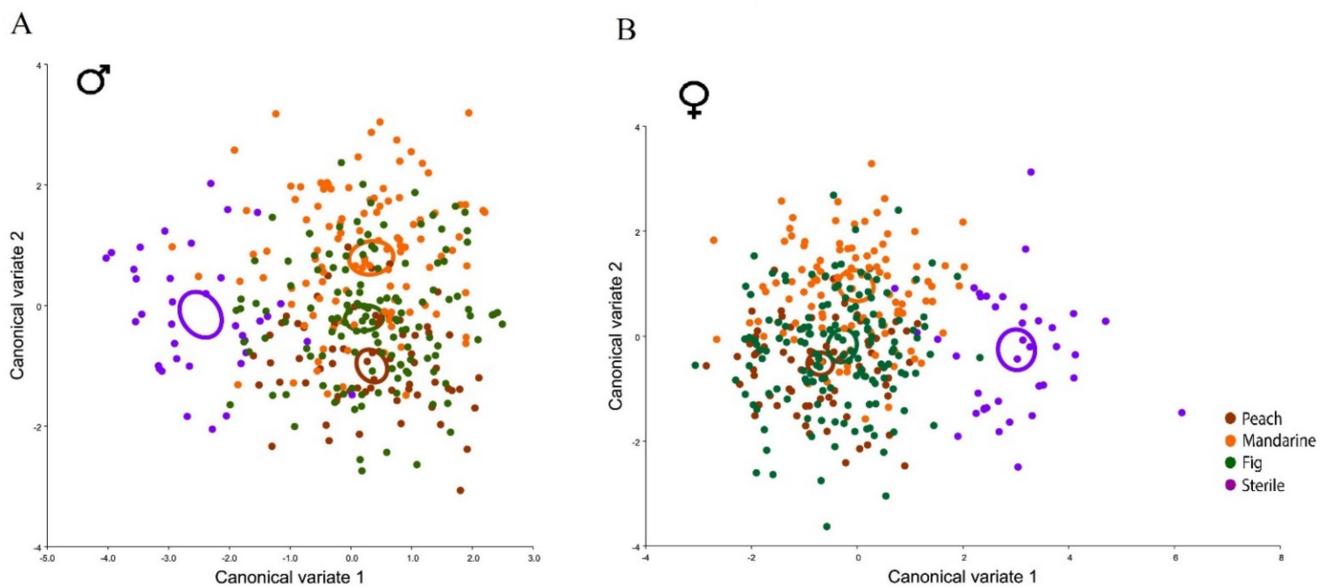


Figure 4. Canonical variate analysis (CVA) of the wings of (A) males and (B) Females of medfly with respect to different hosts (red: peach; orange: mandarin; green: fig; purple: sterile). Ellipses represent the mean values of groups with 90% confidence.

Using the CVA of the wing shape of male and female medflies, the sterile populations and individuals that were bred on mandarins were filtered out compared to the other hosts. Populations that were collected on figs and peaches did not differ from each other. Figure 5A,B shows variability in females and males that were collected from different hosts using the wireframe configuration. In males, the differences are very slight and are evident of the slightly longer wings of the mandarin individuals, which are due to the elongation of the central veins of the wings in the positions of the specific landmarks 4, 5, 8, and 9. In females, the differences in wing shape are more obvious from landmarks 11 and 14 (fig populations) and we can see that the elongated wings of the mandarin flies are due to the elongation of the central veins, as in males, in the positions of the specific landmarks 4, 5, 8, and 9. Such wings are more aerodynamic and allow longer flights. The wings of sterile flies and fig flies are narrow and short, which can be seen from the shortening of the terminal veins at the positions of the specific landmarks 1, 5, 8, 9, and 10–12. Such a wing shape is characteristic of short and slow flights.

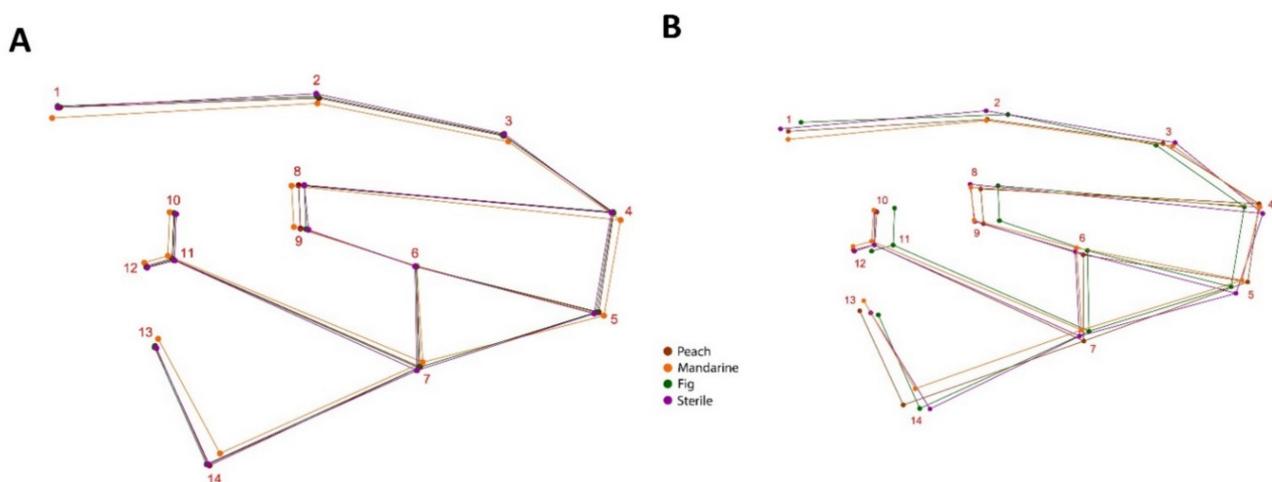


Figure 5. Linear view of the wings of males (A) and females (B) of the medfly from different hosts. The red lines show a schematic shape of a male's peach wing; orange with mandarin; green with figs and purple lines show sterile populations.

Population structure that is based on the Mahalanobis and Procrustes distances between population pairs is highest in sterile populations compared to all other populations (hosts). Very high values for population structure were also found between mandarin populations compared to other hosts (Table 2). Using the Mahalanobis distances between site pairs based on the highest values, we can determine three medfly populations: (1) sterile population; (2) mandarin population; and (3) population from other hosts.

Table 2. Results of CVA analysis with Procrustes and Mahalanobis distances and corresponding *p* values, for males and females between different hosts.

	Peach	Mandarin Mahalanobis Distance <i>p</i> Value	Fig	Peach	Mandarin Procrustes Distance <i>p</i> Value	Fig
MALES						
Mandarin	1.8115 <i>p</i> < 0.0001			0.0099 <i>p</i> < 0.0001		
Fig	1.1727 <i>p</i> < 0.0001	1.2301 <i>p</i> < 0.0001		0.0084 <i>p</i> < 0.0001	0.0054 <i>p</i> = 0.0023	
Sterile	2.9715 <i>p</i> < 0.0001	2.8492 <i>p</i> < 0.0001	2.841 <i>p</i> < 0.0001	0.0178 <i>p</i> < 0.0001	0.0186 <i>p</i> < 0.0001	0.0175 <i>p</i> < 0.0001
FEMALES						
Mandarin	1.6085 <i>p</i> < 0.0001			0.0093 <i>p</i> < 0.0001		
Fig	1.1564 <i>p</i> < 0.0001	1.3039 <i>p</i> < 0.0001		0.0061 <i>p</i> = 0.0014	0.0065 <i>p</i> < 0.0001	
Sterile	3.7637 <i>p</i> < 0.0001	3.3428 <i>p</i> < 0.0001	3.4391 <i>p</i> < 0.0001	0.0229 <i>p</i> < 0.0001	0.0212 <i>p</i> < 0.0001	0.0207 <i>p</i> < 0.0001

Population structure based on Mahalanobis and Procrustes distances between different hosts under the same site showed high values for population structure between all pairwise comparisons (Table 3). Based on Mahalanobis distances, we can determine three medfly populations: (1) a mandarin population; (2) a fig population; and (3) a peach population for both male and female medflies.

Table 3. Results of CVA analysis with Procrustes and Mahalanobis distances and corresponding *p* values, for males and females between different hosts from same location.

	Hvar	Podstrana	Opuzen	Hvar	Podstrana	Opuzen
	Mahalanobis Distance <i>p</i> Value			Procrustes Distance <i>p</i> Value		
MALES						
Mandarin vs. Fig	2.0194 <i>p</i> < 0.0001			0.0093 <i>p</i> = 0.001		
Fig vs. Peach		1.8782 <i>p</i> < 0.0001	1.5926 <i>p</i> = 0.01		0.0095 <i>p</i> = 0.008	0.0078 <i>p</i> = 0.08
FEMALES						
Mandarin vs. Fig	2.1394 <i>p</i> < 0.0001			0.0104 <i>p</i> < 0.0001		
Fig vs. Peach		1.3576 <i>p</i> = 0.06	1.8811 <i>p</i> < 0.0001		0.009 <i>p</i> = 0.006	0.009 <i>p</i> = 0.001

3.2. Variability in the Shape of the Wings of a Medfly from Different Locations

In the canonical variate analysis, females and males were analyzed separately according to the locality from which they originated. Figure 6 shows that the canonical variate analysis of the wing shape of male and female medflies highlights the sterile populations

that were bred in the laboratory and the individuals from Opuzen, compared to other localities. Populations that were collected from the other sites were not different from each other.

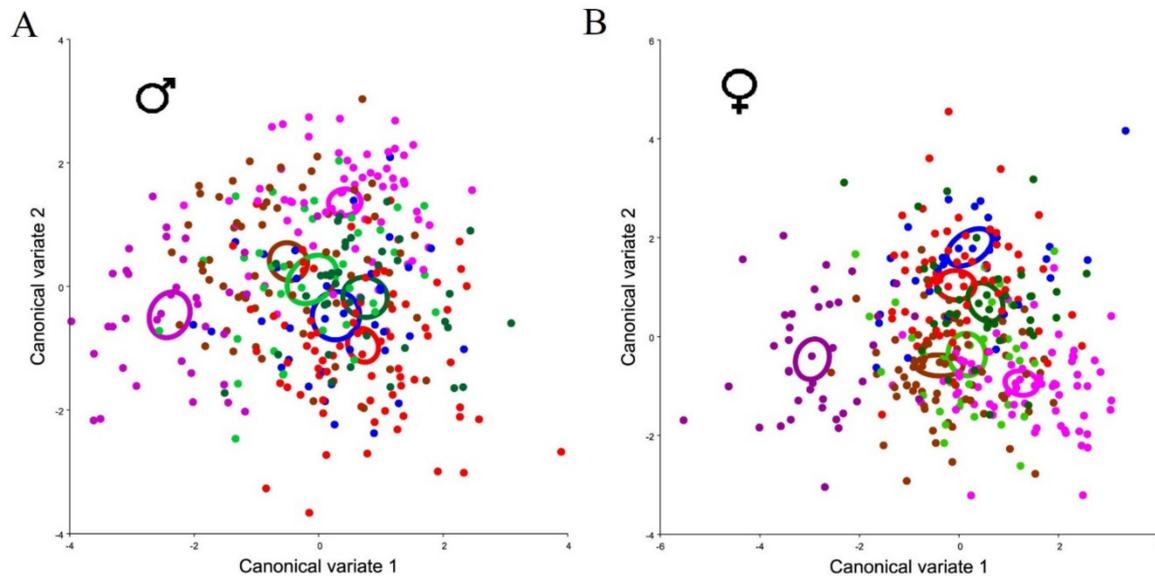


Figure 6. Canonical variate analysis (CVA) of (A): male and (B): female wings of medfly with respect to different collection sites (red: Hvar; blue: Metković; pink: Opuzen; light green: Ston; brown: Podstrana; dark green: Sućuraj; purple: sterile). Ellipses represent the mean values of groups with 90% confidence.

To determine the differences between treatments, CVA showed a clear separation of the laboratory-bred specimens (purple sterile population). IPM and the conventional specimens shared some wing shape characteristics, which are graphically overlaid in the CVA space. The population of SIT was also clearly different in the two sexes (Figure 7).

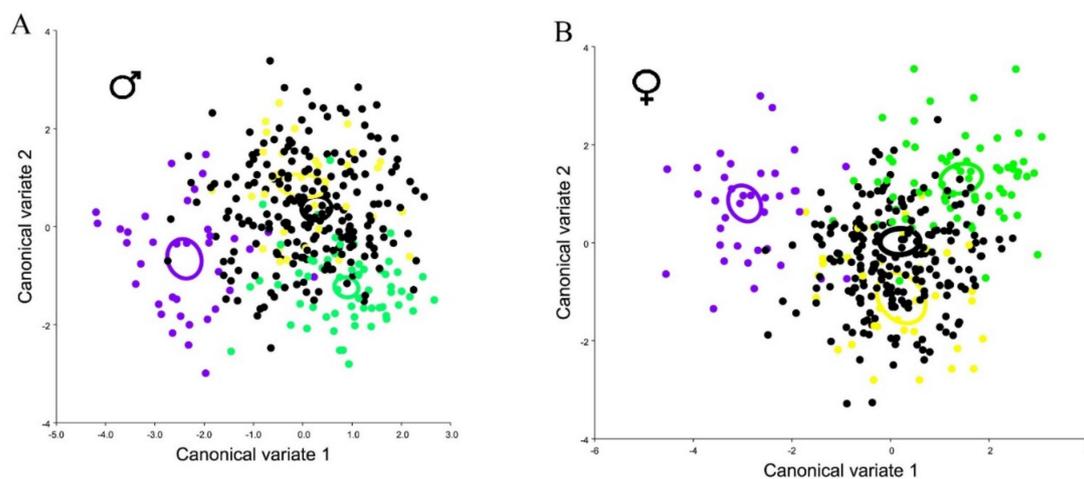


Figure 7. Canonical variate analysis (CVA) of the wings shape between treatments, control samples and laboratory sterile specimens. (A) males and (B) Females of medfly (black: CON, yellow: IPM, green: SIT; purple: sterile). Ellipses represent the mean values of groups with 90% confidence.

The population structure of medfly females based on Mahalanobis and Procrustes distances between site pairs is greatest for the laboratory-bred sterile population and all of the other populations. Very high values for population structure are also found when comparing Opuzen with other sites (Table 4). Using the Mahalanobis distances between

site pairs based on the highest values and CVA, we can determine at least three female populations: (1) a population from the Opuzen site; (2) a sterile population from the laboratory; and (3) a population from the other research sites.

Table 4. Results of CVA analysis with Procrustean and Mahalanobis distances and corresponding p values for females of medfly between different localities.

FEMALE	Hvar	Metković	Opuzen	Ston	Podstrana	Sterile
	Mahalanobis Distance p Value					
Metković	1.4832 $p = 0.0014$					
Opuzen	2.5498 $p < 0.0001$	2.7942 $p < 0.0001$				
Ston	1.9459 $p < 0.0001$	2.3817 $p < 0.0001$	2.0943 $p < 0.0001$			
Podstrana	2.2512 $p < 0.0001$	2.4622 $p < 0.0001$	2.5266 $p < 0.0001$	2.0517 $p < 0.0001$		
Sterile	3.5225 $p < 0.0001$	3.8136 $p < 0.0001$	4.2868 $p < 0.0001$	3.4827 $p < 0.0001$	3.4432 $p < 0.0001$	
Sučuraj	1.4768 $p < 0.0001$	1.7915 $p < 0.0001$	2.2037 $p < 0.0001$	2.2459 $p < 0.0001$	2.3188 $p < 0.0001$	3.8186 $p < 0.0001$
	Procrustes distance p value					
Metković	0.0072 $p = 0.0276$					
Opuzen	0.013 $p < 0.0001$	0.0155 $p < 0.0001$				
Ston	0.0108 $p < 0.0001$	0.0125 $p < 0.0001$	0.0114 $p < 0.0001$			
Podstrana	0.0103 $p < 0.0001$	0.0111 $p < 0.0001$	0.0128 $p < 0.0001$	0.0096 $p < 0.0001$		
Sterile	0.0227 $p < 0.0001$	0.0232 $p < 0.0001$	0.0256 $p < 0.0001$	0.0195 $p < 0.0001$	0.019 $p < 0.0001$	
Sučuraj	0.0074 $p = 0.0061$	0.0084 $p = 0.0126$	0.013 $p < 0.0001$	0.0125 $p < 0.0001$	0.011 $p < 0.0001$	0.0223 $p < 0.0001$

Population structure based on Mahalanobis and Procrustes distances between site pairs is the same as for the female population and is greatest for the laboratory-bred sterile males and all of the other populations. In addition, very high values for population structure were found when the Opuzen site was compared to other sites (Table 5). Using the Mahalanobis distances between site pairs based on the highest values, we can determine at least three male populations: (1) a population from the Opuzen site; (2) a sterile population from the laboratory; and (3) a population from the other research sites.

Table 5. Results of CVA analysis with Procrustean and Mahalanobis distances and corresponding *p* values for males of medfly between different localities.

MALE	Hvar	Metković	Opuzen	Ston	Podstrana	Sterile
	Mahalanobis Distance <i>p</i> Value					
Metković	1.5215 <i>p</i> = 0.0006					
Opuzen	2.3725 <i>p</i> < 0.0001	2.2746 <i>p</i> < 0.0001				
Ston	1.837 <i>p</i> < 0.0001	1.4733 <i>p</i> = 0.0589	1.8769 <i>p</i> < 0.0001			
Podstrana	2.224 <i>p</i> < 0.0001	1.8551 <i>p</i> < 0.0001	2.1022 <i>p</i> < 0.0001	1.7606 <i>p</i> < 0.0001		
Sterile	3.1992 <i>p</i> < 0.0001	3.0333 <i>p</i> < 0.0001	3.3603 <i>p</i> < 0.0001	2.7428 <i>p</i> < 0.0001	2.7072 <i>p</i> < 0.0001	
Sučuraj	1.3312 <i>p</i> = 0.0023	1.6089 <i>p</i> = 0.0045	1.0232 <i>p</i> < 0.0001	1.8999 <i>p</i> < 0.0001	1.9145 <i>p</i> < 0.0001	3.3065 <i>p</i> < 0.0001
	Procrustes distance <i>p</i> value					
Metković	0.0072 <i>p</i> = 0.037					
Opuzen	0.0139 <i>p</i> < 0.0001	0.0113 <i>p</i> < 0.0001				
Ston	0.0112 <i>p</i> < 0.0001	0.0071 <i>p</i> = 0.2187	0.0078 <i>p</i> = 0.0035			
Podstrana	0.0117 <i>p</i> < 0.0001	0.008 <i>p</i> = 0.414	0.0109 <i>p</i> < 0.0001	0.0081 <i>p</i> = 0.0097		
Sterile	0.0209 <i>p</i> < 0.0001	0.0187 <i>p</i> < 0.0001	0.0178 <i>p</i> < 0.0001	0.0158 <i>p</i> < 0.0001	0.0172 <i>p</i> < 0.0001	
Sučuraj	0.0069 <i>p</i> = 0.0291	0.0082 <i>p</i> = 0.0745	0.0113 <i>p</i> < 0.0001	0.0103 <i>p</i> = 0.0008	0.0096 <i>p</i> = 0.0009	0.0195 <i>p</i> < 0.0001

4. Discussion

This research is the first of its kind to use geometric morphometric methods in the study of the medfly in order to determine the influence of the host plant on the potential invasiveness and adaptability of this pest. In addition to high biotic potential and reproductive capacity [3,18], the variability of the pest that develops as a result of exposure to different agroecological growing conditions is one of the key components for the successful adaptation and establishment of the species in new areas [9,27–30,59]. This study revealed that: (1) wing-shaped sexual dimorphism is present in all of the medfly populations that were studied; (2) the hosts in which the medfly develops influence the shape of the wing or condition its variability; (3) there is considerable variability between sterile and wild individuals in male and female populations; (4) there is a high phenotypic plasticity of medfly populations in the study area.

A standard geometric morphometric analysis revealed significant wing-shaped variability between all of the females and males in the populations that were studied. Wing-shaped sexual dimorphism has been very successfully demonstrated using geometric morphometrics in various insect species, from the orders Diptera, Coleoptera, Hymenoptera and Lepidoptera [49,52,60–63]. In this study, females were found to have more elongated and larger wings compared to males, which is a confirmation of the previous study by Lemic et al. [52]. Elongated wings are considered to be more aerodynamic and less prone to flight damage [46,64]. The elongated wing shape helps females in migratory flights and in locating oviposition sites [65]. Isard et al. [66] found that the flight duration of western corn borer females increases before oviposition. The extended wings are also considered to be an advantage in oviposition [65]. Studies on the western corn rootworm have shown

that the long flights cease after the egg-laying ceases [66]. In some insect species, such as the potato moth, males have been found to have longer wings that allow them to fly long distances in search of females and food [67]. The males medflies do not need long flights because the males release pheromones that attract females, which fly longer distances to find them, mate with them, and ensure the continuation of the species [68]. It is evident that female medflies have a greater need for more frequent and longer flights over a longer period of time, making them the responsible sex for the expansion into new territories.

In this study, the populations of the medfly that were developed in mandarin fruit differed markedly from the flies that were collected in peaches or figs. In the males, these differences were very slight and were manifested as slightly longer wings of the mandarin individuals, which is due to the elongation of the central veins of the wings in the positions of the specific landmarks 4, 5, 8, and 9. In females, the differences in wing shape were much greater and we can see that the elongated wings of mandarin flies are due to the elongation of the central veins at the same specific landmarks as in the males (4, 5, 8, and 9). As mentioned in the previous section, elongated wings are characteristic of individuals that perform migratory flights, especially with the aim of laying eggs [65]. Temperature and the food on which larvae feed are important factors that alter insect body size and, consequently, wing size [69,70]. In most ectothermic organisms, lower temperature slows development and increases body size [71]. The quality (expressed by chemical composition) of the plant host in which eggs and larvae develop also affects the body size of adult forms. Plant hosts with poorer nutritional value can interact with temperature in order to alter the norms of the thermal reactions in the insect body, leading to a reduction in insect body size [72]. Iglesfield [73] demonstrated that the eggs of the medflies that were developed in oranges produced larger adult flies than those that were developed in pears. In a study by Navarro-Campos et al. [16], larger adult medflies were found to develop from larvae that were exposed to lower temperatures. The same authors observed larger adult flies developing from infested fruits that were collected in October compared to those that were collected in August and September, when temperatures were significantly higher. In this study, fruits with medfly larvae were collected during a period with similar temperature conditions. Therefore, we can conclude that temperature is not the cause of the demonstrated variability in the mandarin populations. The nutritional value of all the fruits that were studied is very similar, the content of protein, fat and fiber is almost identical in all three host fruits, while figs have a slightly higher concentration of sugars [74–76]. What distinguishes mandarins from peach and fig fruits is the large amount of aspartic acid, of which there is over 130 mg in 100 g of mandarin [76]. This acid is involved in protein synthesis, growth hormone synthesis, and assists in the process of converting carbohydrates into energy [77]. Therefore, we can speculate that aspartic acid may be a possible reason for the larger wings and thus the greater variability of the mandarin medflies compared to other hosts in the study. Future estimates of medfly adaptation to new hosts should consider the amount of aspartic acid as a possible factor conditioning the evolution of individuals with higher flight capabilities and thus greater invasiveness.

Sterile Insect Technique (SIT) is an environmentally friendly and species-specific control method for Tephritidae fruit flies and various other Diptera, Lepidoptera and Coleoptera agricultural pests. SIT is based on the release of a large number of sterile males that compete with wild males and disrupt the copulation process of wild pest populations, which affects reproduction and reduces the size of wild populations [14]. This technique has been found to be very effective when applied area-wide, in larger and isolated geographical areas along with other cultural (fruit sanitation) and IPM (mass trapping, attracting and killing) methods. Isolated areas such as the Neretva River valley, where SIT has been successfully used to control medfly for more than ten years without the use of insecticides [78], were included as a source of medfly populations (Opuzen site). Since the females choose between sterile males and wild males, which are more dominant in size and better at courtship, the use of SIT is further complicated [79,80]. De

Aquino et al. [80] have shown that females choose larger males for copulation even when the ratio of small to large males is 5:1. The ability to court males, along with their size, is a critical factor in the choice of a mate for females during copulation. In addition, males from wild populations have been shown to have better courtship abilities and females are more likely to select wild males for copulation, although the sterile males that were reared in the laboratory (depending on larval food quality, density of larvae during rearing, etc.) may be more dominant in size [80]. The results of this study show considerable variability in the sterile compared to the wild populations of medflies. Individuals of both sexes of the sterile population have smaller and narrower wings compared to all of the wild populations that were studied. The wings of the sterile flies are narrow and short, which is due to a shortening of the veins at the positions of the specific landmarks 1, 5, 8, 9, and 10–12. These points are located on the radial vessels and on the medial and anal vessels, which are an important anatomical feature for distinguishing morphotypes based on different wings of species from the fruit fly family [81]. It is not known what part of the process of mass rearing under laboratory conditions and/or sterilization procedures causes such morphological changes, but it is certain that they make the sterile individuals less competitive after their release under natural conditions [82]. Since the competitiveness of sterile individuals in release areas is very low, the number of sterile male insects required per unit of area to achieve an appropriate ratio of sterile to wild male insects should be estimated based on the absolute population density of the pest in the target area [83].

This study shows some variability in the populations in the studied area, caused primarily by host plant species, which is the result of the development of localized phenotypic plasticity in the population. Phenotypic plasticity is defined as a change in phenotypic expression of a genotype in response to agroecological factors [33] and has been shown to have significant evolutionary consequences [33,34]. In previous studies by Lemic et al. [52], no clear variability was found between populations from the narrower area in southern Dalmatia. Gaspa-rich et al. [84] and Gasperi et al. [8] also found a low population structure of medflies using biochemical and molecular markers. According to the results of these authors, populations of medflies are genetically similar throughout the Mediterranean region. Many studies suggest that newly invasive species exhibit a higher degree of phenotypic plasticity, but empirical tests of this theory are very rare [85,86]. Although the medfly is considered an invasive species, it has been present in Croatian coastal areas for more than fifty years [87]. During this time, the medfly has successfully adapted to numerous hosts (apricot, peach, mandarin, nectarine, plum, fig, orange, apple, pear, etc.) in the area where the samples for this study were collected [14]. The detected but low morphological variability between populations from apparently very different agroecological systems and different host plants that was investigated in this study indicates the stability of the medfly genotype, which is reflected in a stable phenotype (explained in Bouyer et al. [43]). Low variability, which is manifested as sexual dimorphism and dependent on the type of cultivation (conventional, IPM and SIT) and the host plant, is characteristic of invasive species living under changing environmental conditions. Contrary to expectations, the study observed the variability (structure) of populations that originated from the sampling site in the Opuzen area compared to other geographical sites from which the sampled populations were taken. Since medfly control strategies are applied in the fruit growing areas near the town of Opuzen, which has precluded the use of conventional methods for a decade [78,88], abiotic changes in medfly habitat conditions in this area are a possible cause of the observed variability of this population compared to other sites that are mainly represented by conventional control methods. Considering the high prevalence and invasiveness of the medfly, as well as its proven ability to adapt to different agroecological conditions (phenotypic plasticity), it is expected that the medfly will spread and adapt to previously uninfected areas and new hosts during periods of significant climatic change.

5. Conclusions

The female medflies have more elongated and larger wings compared to the males. These wings are more aerodynamic and less prone to flight damage. This allows the females to fly more frequently and for longer periods of time, making them responsible for dispersal into new areas. It has been shown that the wing shape of adult medflies from mandarin fruit varies compared to adult flies from peaches and figs. Aspartic acid could be a possible reason for the larger wings and variability of the mandarin medfly compared to other hosts. The wings of sterile flies (of both sexes) are narrow and short, resulting in the very poor flight ability of these individuals and the lower competitive ability of sterile males compared to wild males. The demonstrated but relatively low morphological variability between the populations from different agroecological systems and host plants indicates the stability of the medfly genotype, which is reflected in the demonstrated stable phenotype (phenotypic plasticity). The high phenotypic plasticity of the fly is a response to the high adaptability of this organism to agroecological development factors, which is characteristic of its invasiveness and ability to rapidly adapt the genotype to environmental changes, making the medfly a threat to new, uninfected areas and new hosts.

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