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Patterns of asymmetry in wing traits of three island and one continental population of *Merodon albifrons* (Diptera, Syrphidae) from Greece

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ABSTRACT

Background: Individuals of populations experiencing environmental and/or genetic disturbance during their development experience a reduction in buffering capacity of the developmental system. Such a reduction influences the level of developmental stability, which is often reflected in morphological traits such as asymmetry. Three kinds of asymmetry have been described: directional asymmetry (DA; i.e. left and right body side consistently differ from each other), fluctuating asymmetry (FA; i.e. subtle non-directional differences between left and right sides in bilaterally symmetrical traits), and antisymmetry (i.e. deviation from symmetry towards either the right or left sides). Fluctuating asymmetry is often used as an indicator of developmental stress. The variation of FA level is considered to be taxon, trait and stress specific. Accordingly, being exposed to various forms of environmental and/or genetic stress, the levels of FA may differ among demes. Moreover, different genetic constraints of two wing traits (lower heritability of wing size vs. higher heritability of wing shape) may influence the difference between island and mainland populations in the level of size FA would be more prominent than in the level of shape FA.

Hypothesis: Island populations, which are often isolated and small, operate different evolutionary processes affecting developmental processes from those present in mainland populations. Hence, island populations experience significantly different FA compared with continental populations.

Organism: The hoverfly, *Merodon albifrons* (Diptera, Syrphidae)

Times and places: We collected a total of 60 female and 141 male specimens in spring 2009, 2010, and 2011 from the Greek islands of Lesbos (25 females and 38 males; May 2009), Chios (11 females and 60 males; May 2009), and Naxos (12 females and 13 males; May 2010). We also collected from the Greek mainland locality of Volos (12 females and 30 males; May 2009 and May 2011).

Analytical methods: We used landmark-based geometric morphometrics to analyse level and patterns of asymmetry (FA, DA, and antisymmetry) in two wing traits (size and shape) within/among populations. We completed morphometric and statistical analyses using the MorphoJ package (version 1.04a) (Klingenberg, 2011), R software 2.15.1 (R Development Core Team, 2008),

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and Statistica 10 (StatSoft, 2012). Since male and female wing shape asymmetries were not significantly different, we pooled the sexes in all analyses.

Results: We found highly significant FA, DA, and differences among individuals in wing size and shape in all studied samples. Antisymmetry was absent from the data. We found no significant difference in FA level in wing size (Levene's test: $F_{2,156} = 2.11$, $P = 0.12$) and shape (Levene's test: $F_{2,156} = 2.40$, $P = 0.09$) among three island populations, or in wing shape (Levene's test: $F_{1,199} = 0.55$, $P = 0.46$) between island and mainland populations. In contrast, the level of FA in wing size was significantly different (Levene's test: $F_{1,199} = 14.01$, $P < 0.001$) between island and mainland populations. The pattern of FA shape variation measured by matrix correlation and visualized using principal component analysis suggested similarities among the populations analysed. In all populations, landmarks located in the proximal part of the wing (wing base) contributed substantially to overall shape variability, which reflected a response of the same developmental processes to disturbance during the development of individuals.

Conclusion: Populations from the island and mainland localities are connected, resulting in a common pattern of wing shape development.

Keywords: developmental instability, fluctuating asymmetry, geometric morphometrics, *Merodon albifrons*, wing size and shape.

INTRODUCTION

Islands have been considered as 'natural experiments' (e.g. Dennis *et al.*, 2000; Poulakakis *et al.*, 2003; Comes *et al.*, 2008; Georghiou and Delipetrou, 2010; Désamoré *et al.*, 2012; Simaiakis *et al.*, 2012), and thus natural populations are important models for studying microevolutionary dynamics (Whittaker and Fernández-Palacios, 2007). The role of islands in diversification of biological diversity is highlighted, especially in the light of conservation biology (e.g. Whittaker and Fernández-Palacios, 2007; Dapporto and Dennis, 2008; Wilson *et al.*, 2009; Dennis *et al.*, 2012). Researchers of phenotypic and genetic diversity have recently focused on the conservation implications of these patterns and the processes underlying them (Dapporto and Dennis, 2008; Dennis *et al.*, 2012). For instance, it is well documented that vulnerability to extinction is linked to a decrease of genetic diversity, and consequently fitness and evolutionary potential (Amos and Balmford, 2001; Crnokrak and Barrett, 2002; Reed and Frankham, 2003, Pertoldi *et al.*, 2007).

During environmental perturbations, organisms are exposed to stress, but their response depends on their own evolutionary potential and genetic diversity (e.g. Hedrick and Kalinowski, 2000; Armbruster and Reed, 2005; Pertoldi *et al.*, 2006). Given that organisms experience different changes in their environment, which has an impact on their adaptation and survival, researchers have studied phenotypic variation of functionally important organs (such as wings) to elucidate the role of developmental processes and the potential for adaptive evolution (Willmore *et al.*, 2007). Indeed, development mediates complex interactions between genetic and environmental factors affecting the shape of morphological structures, which in turn are subject to natural selection (Klingenberg, 2010). Individuals experiencing environmental and/or genetic disturbance during their development are affected by a reduction in buffering capacity of the developmental system (Klingenberg and Nijhout, 1999; Lens *et al.*, 2002; Klingenberg, 2003). Because developmental stability is a reflection of buffering capacity to produce an accurate replication of a phenotype, its assessment is usually undertaken using patterns of asymmetry of such replications (Palmer and Strobeck, 1992; Stige *et al.*, 2006). Three kinds of asymmetry have been described: directional asymmetry (DA; i.e. left and right body side consistently differ from each other), fluctuating asymmetry (FA; i.e. subtle non-directional differences between left and right sides in bilaterally symmetrical traits), and antisymmetry (i.e. directional deviation from symmetry towards either the right or left sides). Because FA represents non-directional differences between replications, the use of FA variance between bilaterally symmetric traits has been considered a measure of developmental stability. Indeed, FA has been shown to be a measure of stress and fitness, and to be related to the evolutionary potential of traits (Lens *et al.*, 2002; Van Dongen *et al.*, 2009). In addition, especially in conservation ecology, FA has been proposed as an early warning tool in conservation planning (Lens and Van Dongen, 2002; Lens *et al.*, 2002).

In insects, wings have been considered valuable for the study of systematics and taxonomy (e.g. Aytekin *et al.*, 2007; Francuski *et al.*, 2009; Baracchi *et al.*, 2011). Furthermore, wing shape and size have been shown to be sensitive characters and hence have been considered to be valuable traits in the study of developmental processes (Gilchrist *et al.*, 2000) and adaptive evolution, owing to their importance for flight and thus fitness (Kölliker-Ott *et al.*, 2003). For example, in *Triatoma dimidiata* it was observed that individuals with large and more symmetric wings had greater dispersal potential (Nouvellet *et al.*, 2011). However, studies exploring the level of FA in island and continental populations of both invertebrates and vertebrates are limited (e.g. Juste *et al.*, 2001; Camara *et al.*, 2006; White and Searle, 2008; Bancila *et al.*, 2010). Previous studies of wing FA in hoverfly taxa included populations of the northern species, *Cheilosia naruska* and *C. longula*, for which significant FA and DA of both wing size and shape were observed (Milankov *et al.*, 2010a, 2010b).

We thus chose to study natural populations of *Merodon albifrons* Meigen, 1822 (Diptera, Syrphidae), originating from three Greek islands of the Aegean archipelago (Chios, Lesbos, and Naxos) and one mainland population (Volos; Greece). Lesbos and Chios are the largest and fifth largest island, respectively, of a series of North Aegean islands situated in the Aegean Sea along the west coast of Turkey, while Naxos is the largest island in the Cyclades island group of the Aegean archipelago. The mainland locality of Volos is located at the foot of the peninsula of Pelion in the centre of Greece. All sample sites are characterized by different geological history and ecological heterogeneity (Comes *et al.*, 2008). Systematic and taxonomic studies of some hoverfly taxa based on molecular markers (mitochondrial DNA) and morphological characters revealed a remarkable biodiversity of hoverflies on the Aegean archipelago (Vujić *et al.*, 2007; Ståhls *et al.*, 2009; Petanidou *et al.*, 2011; Radenković *et al.*, 2011).

The hoverfly *Merodon albifrons* prefers open ground, stony, unimproved, non-calcareous dry grassland and open, tall herb areas in *Quercus pubescens* and *Q. ilex* forest on calcareous substrates (Speight, 2011). Adults visit flowers of *Urginea maritime* (Standfuss and Claussen, 2007), *Chrysanthemum*, *Euphorbia*, *Helianthemum*, *Ranunculus*, and *Senecio* (Speight, 2011). The species is widely distributed, from central France south to the Mediterranean and North Africa, and from western Spain eastwards through central and southern Europe to the Crimea and Azerbaijan (Speight, 2011). *Merodon albifrons* is relatively abundant on the islands. Since the Aegean islands, as a part of the Mediterranean basin, represent a centre of species diversity and endemicity (Medail and Diadema, 2009), in a previous study using the integrative approach (including nuclear allozyme loci, COI mtDNA and wing traits, i.e. shape and size) we evaluated spatial and temporal patterns of diversity of *M. albifrons* (V. Milankov *et al.*, unpublished manuscript). Assuming that different evolutionary processes operate on island populations versus mainland populations, in this study we used FA as a tool to infer developmental instability that contributes to phenotypic variation of traits of adaptive importance. Thus, by applying the geometric morphometric approach and multivariate

Table 1. Origins and sample sizes of *Merodon albifrons* populations

Location	Longitude/latitude	Collection date	Males	Females	Total
Lesbos	26°16′E, 39°16′N	15–16 May 2009	38	25	63
Chios	26°07′E, 38°23′N	13–14 May 2009	60	11	71
Naxos	25°28′E, 37°05′N	12 May 2010	13	12	25
Volos	22°56′E, 39°22′N	09 May 2009, 25 May 2011	30	12	42

Note: Collections on the islands were made by V. Milankov, E. Rättel and G. Ståhls; those on the mainland at Volos by M. Đurakić, Lj. Francuski and K. Standfuss.

statistics, we explored the within-individual variation (FA) in wing size and shape of the studied hoverfly populations. Given fluctuating asymmetry in wing size and shape, we furthermore wished to determine whether the amount of FA and pattern of left–right displacement of landmarks differ significantly among island samples as well as among island and mainland populations.

METHODS AND MATERIALS

Sample collection

In total, 201 specimens (141 males and 60 females) of *Merodon albifrons* were collected from three islands (Lesbos, Chios, and Naxos) and one mainland location (Volos) in Greece (Table 1, Fig. 1). The sample used in this study has previously been subjected to analysis of genetic diversity (using allozyme loci and COI mtDNA) and phenotypic variation (geometric morphometric analysis of wing size and shape) (V. Milankov *et al.*, unpublished manuscript).

Geometric morphometrics

To analyse patterns of wing size and shape asymmetry, we employed landmark-based geometric morphometrics. The right and left wings of 60 females and 141 males of *M. albifrons* were removed and mounted in Hoyer's medium between microscope slides. Wing images were captured using a digital camera (Leica DFC320) connected to a stereomicroscope (Leica MZ12.5). Sixteen landmarks positioned at vein intersections or terminations (Fig. 2) were collected using TpsDig 2.16 (Rohlf, 2010) and expressed as *x,y*-coordinates in Cartesian space. To quantify and minimize measurement error, all wings were digitized three times by the same person (J.L.). From landmark coordinates, centroid size (the square root of the sum of squared distance between each landmark and the wing centroid) and shape information (Procrustes coordinates) were extracted using a full Procrustes fit (Klingenberg and McIntyre, 1998). Given that hoverfly wings have matching symmetry, shape variation was separated into a symmetric component (among-individual variation) and asymmetric component (within-individual variation) (Klingenberg *et al.*, 2002).

Statistical analysis

Asymmetry in wing size and shape was investigated following Klingenberg and McIntyre (1998). Procrustes ANOVA on centroid size and Procrustes coordinates were used to assess the contribution of individual variation, fluctuating asymmetry (FA), directional

Fig. 1. Map of population sampling locations from Greece: (1) Lesbos; (2) Chios; (3) Naxos; (4) Volos.

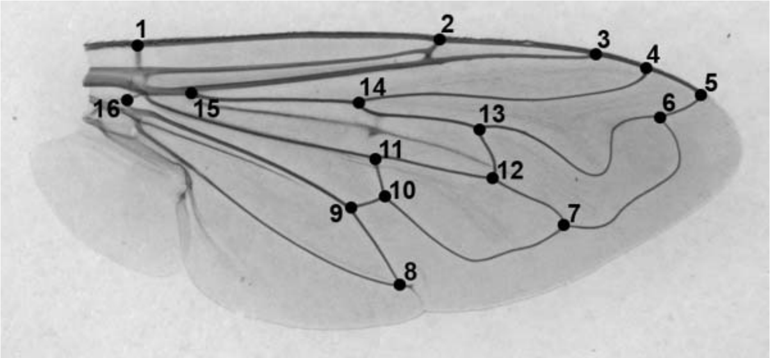

Fig. 2. The locations of 16 landmarks selected for geometric morphometric analysis of *M. albifrons*.

asymmetry (DA), and measurement error (E) to the overall variation. In the model implemented here, 'Individuals' (I) is a random factor that assesses variation among individuals, 'Sides' (S) is a fixed factor that assesses directional asymmetry (DA), the Individuals × Sides interaction (I × S) assesses fluctuating asymmetry (FA), and the measurement error (E) assesses variation in the replicate measurements (Leamy, 1984; Palmer, 1994). Prior to calculation of measurement error and asymmetry, the data were checked for antisymmetry, i.e. deviation from symmetry towards either the right or left sides. We used the Kolmogorov-Smirnov test to test for deviation of signed right–left differences from normality.

To measure the amount of FA, we calculated variance of both size (FA centroid size) and shape. The difference in the amount of FA in size and shape was tested with a Levene's test of homogeneity of variances. The FA of centroid size was quantified using signed right–left difference in wing size. Shape asymmetry was quantified using both Procrustes and Mahalanobis distances of each observation from the mean shape of its respective group. These two distances quantify shape variation differently (for details, see Klingenberg and Monteiro, 2005, and references therein). Procrustes distance is a measure of absolute shape differences and treats shape deviations from the sample mean equally, regardless of their direction (isotropic model) (Dryden and Mardia, 1998). Mahalanobis distance measures the differences between groups relative to the within-group variation and therefore accounts for the group-specific direction of shape variation (e.g. Mardia *et al.*, 1979). Because we were interested in the difference in the amounts of variation between islands as well as between islands and the mainland, we calculated Mahalanobis distance in two ways depending on the level of comparison. When assessing differences among islands, Mahalanobis distances were calculated using the pooled covariance matrix of the respective island. For the islands vs. mainland comparison, three island populations were pooled into one sample, and therefore we used the pooled covariance matrix of all islands to calculate Mahalanobis distances at this level of comparison. In addition, we tested for differences in mean shape asymmetries. A permutation test with 10,000 iterations was used to assess statistical significance against the null hypothesis of equal group shape means. We tested for differences between *a priori* defined groups according to geographic position (island populations vs. mainland population).

To observe the association of size and shape variation, we employed two methods. First, we compared the correlation between individual values of centroid size asymmetry and the univariate estimator of shape asymmetry (Procrustes and Mahalanobis distances) (Breuker *et al.*, 2006). This was done for each population. Second, we analysed allometry to determine whether size had an effect on shape asymmetry using multivariate regression, treating shape completely in the multivariate context. Shape asymmetry (asymmetric component) was regressed onto signed asymmetry of centroid size. The statistical significance of regression was estimated using the permutation test with 10,000 iterations against the null hypothesis of independence between size and shape.

The pattern of FA shape variation among the three island populations, as well as between the island and mainland populations, was also compared with matrix correlation between covariance matrices. Matrix correlation was calculated with (full variance–covariance matrix) and without (matrix constituted of covariance alone) the diagonal blocks of the covariance matrices and tested against the null hypothesis of complete dissimilarity with 10,000 random permutations of landmarks. Furthermore, to analyse and display the pattern of variation of asymmetry in the positions of landmarks throughout the wing, we used principal components analysis (PCA) on asymmetric components of shape variation.

Since in all four populations we found that allometry contributed to the asymmetry (significant dependence between size and shape), residuals from the multivariate regressions were used in subsequent analyses of landmark variation. To visualize the shape variation, we used the first principal component (PC1) since it accounted for most of the variation in the original landmark configuration compared with the other principal components (26–42%). To determine whether and to what extent asymmetry shape changes were concordant with shape changes concerning sex dimorphism and inter-population differentiation, we applied PCA to the data set used in our previous study of the distribution of molecular and phenotypic diversity of *M. albifrons* that contained landmark configurations of the right wing (V. Milankov *et al.*, unpublished manuscript). From that data set, we used the spring generation of the Greek populations, and omitted the autumn generation from Lesbos and Chios islands and both generations from Spain.

Morphometric and statistical analyses were conducted using the MorphoJ package (Klingenberg, 2011), Statistica 10 (StatSoft, 2011), and R software v.2.15.1 (R Development Core Team, 2008).

RESULTS

Permutation tests revealed no differences in asymmetry between females' and males' mean shapes (Procrustes distance 0.003, $P = 0.042$; Mahalanobis distances 0.948, $P = 0.28$). Also, two-way MANOVA showed that the amount of shape FA was slightly different between the sexes (Wilks' $\lambda = 0.78$; $F_{28,166} = 1.72$; $P = 0.02$) as well as populations (Wilks' $\lambda = 0.37$; $F_{84,497.5} = 2.33$; $P < 0.001$). However, Wilks' λ was two-fold higher for the sex factor compared with the population factor, indicating that sexual dimorphism in asymmetry was negligible in population comparisons. Thus, in subsequent analyses we pooled the sexes.

Measurement error and antisymmetry

Procrustes ANOVA on wing size and shape was performed for each population and results showed highly significant FA (Individual × Side interaction), DA (Side effect), and difference among individuals in the three island populations (Chios, Lesbos, and Naxos) and one mainland (Volos) population (Table 2). Also, the measurement error for wing size and shape was estimated and mean squares of FA, DA, and individual variation were found to exceed the error component, indicating that the contribution of measurement error to overall shape variation was small. Furthermore, a Kolmogorov-Smirnov test demonstrated that signed difference between right and left side wings did not depart significantly from normality, reflecting an absence of antisymmetry in the data. Thus, we focused on the study of wing size and shape FA.

Amounts of fluctuating asymmetry

For centroid size, Levene's test revealed a non-significant difference in the amount of FA among island populations ($F_{2,156} = 2.11$, $P = 0.12$), while island size FA was significantly different from that of the mainland population (Volos) ($F_{1,199} = 14.01$, $P < 0.001$). The contribution of size FA variation compared with other sources of variation was less in the island populations (overall 0.33%, Chios 0.23%, Lesbos 0.49%, Naxos 0.16%) than the mainland population (0.67%).

Table 2. Procrustes ANOVA of centroid size (CS) and wing shape (SH) for four populations of *M. albifrons*

	Effect	SS	MS	d.f.	*F*	*P*
Lesbos						
CS	I	1581663.4045	25510.7001	62	200.25	<0.0001
	S	9048.3235	9048.3235	1	71.03	<0.0001
	I × S	7898.5095	127.3953	62	54.39	<0.0001
	E	590.2983	2.3425	252		
SH	I	0.13981088	0.00008054	1736	8.19	<0.0001
	S	0.00179338	0.00006405	28	6.51	<0.0001
	I × S	0.01707753	0.00000984	1736	22.61	<0.0001
	E	0.00306996	0.00000044	7056		
Chios						
CS	I	2892176.6686	41316.8096	70	429.32	<0.0001
	S	4007.2062	4007.2062	1	41.64	<0.0001
	I × S	6736.6830	96.2383	70	48.45	<0.0001
	E	560.6175	1.9740	284		
SH	I	0.14402542	0.00007348	1960	8.58	<0.0001
	S	0.00092824	0.00003315	28	3.87	<0.0001
	I × S	0.01679549	0.00000857	1960	20.24	<0.0001
	E	0.00336686	0.00000042	7952		
Naxos						
CS	I	1120654.8256	46693.9511	24	623.42	<0.0001
	S	10797.7689	10797.7689	1	144.16	<0.0001
	I × S	1797.5794	74.8991	24	33.72	<0.0001
	E	222.1486	2.2215	100		
SH	I	0.05675647	0.00008446	672	13.22	<0.0001
	S	0.00316645	0.00011309	28	17.70	<0.0001
	I × S	0.00429311	0.00000639	672	18.31	<0.0001
	E	0.00097716	0.00000035	2800		
Volos						
CS	I	1401670.3119	34187.0808	41	145.58	<0.0001
	S	15546.0873	15546.0873	1	66.20	<0.0001
	I × S	9628.0400	234.8302	41	136.61	<0.0001
	E	288.7914	1.7190	168		
SH	I	0.10915734	0.00009508	1148	11.02	<0.0001
	S	0.00171980	0.00006142	28	7.12	<0.0001
	I × S	0.00990378	0.00000863	1148	24.32	<0.0001
	E	0.00166841	0.00000035	4704		

Note: SS = sum of squares; MS = mean squares; d.f. = degree of freedom.

We calculated Procrustes and Mahalanobis distance and the variances based on the two distance measures to determine the amount of shape variation. These two measurements of shape FA were strongly correlated in Chios ($r = 0.59$, $P < 0.001$), Lesbos ($r = 0.50$, $P < 0.001$), and Volos ($r = 0.63$, $P < 0.001$) but not Naxos ($r = 0.33$, $P = 0.11$). Such strong correlations indicate that Procrustes and Mahalanobis distance could be considered equivalent measures of shape variation (Breuker *et al.*, 2006).

Comparing the amount of FA among three island populations, the inter-population difference in shape FA was non-significant when Procrustes distance was used ($F_{2,156} = 2.40$, $P = 0.09$) but significant with Mahalanobis distance ($F_{2,156} = 15.93$, $P < 0.001$). Because of the lack of correlation between Procrustes and Mahalanobis distances, we further excluded Naxos, and found that population difference in shape FA using Mahalanobis distance between the Lesbos and Chios populations was non-significant ($F_{2,132} = 3.31$, $P = 0.07$). In the next step, we compared shape FA of island populations (a matrix was generated for the sample consisting of island populations pooled together) with that of the mainland population (Volos) and observed non-significant differences in the amount of FA with Procrustes distance ($F_{1,199} = 0.55$, $P = 0.46$) but significant differences with Mahalanobis distance ($F_{1,199} = 10.58$, $P < 0.01$). Once again, the amount of shape FA obtained based on Mahalanobis distance did not differ significantly between island and mainland samples ($F_{1,174} = 0.99$, $P = 0.32$) when the Naxos population was omitted. However, a permutation test revealed a small but significant difference between the mean shape configurations of island populations and the mainland population (Procrustes distance: 0.0034, $P = 0.04$; Mahalanobis distance: 1.5471, $P < 0.001$). Shape FA contributed 8.1% and 9.4% to total shape variation in mainland and island populations (Chios 10.1%, Lesbos 10.6%, Naxos 6.6%), respectively.

Associations of size and shape variation

Correlations between the amount of shape FA and centroid size FA were not significant in any of the populations when both Procrustes distance (Chios: $r = -0.03$, $P = 0.81$; Lesbos: $r = 0.09$, $P = 0.47$; Naxos: $r = 0.38$, $P = 0.06$; Volos: $r = 0.09$, $P = 0.55$) and Mahalanobis distance (Chios: $r = -0.04$, $P = 0.73$; Lesbos: $r = 0.08$, $P = 0.55$; Naxos: $r = 0.002$, $P = 0.99$; Volos: $r = 0.25$, $P = 0.11$) were used as shape measures. However, in all four populations a significant effect of size on shape asymmetry (allometric effect) was observed. In island populations, asymmetry of size accounted for 13.98% in asymmetry of shape in Lesbos ($P < 0.001$), 8.08% in Naxos ($P < 0.05$), 6.88% in Chios ($P < 0.001$) , and 13.78% ($P < 0.001$) in the mainland population of Volos.

Patterns of shape variation

Comparison of covariance matrices of three island populations revealed significant matrix correlation ($P < 0.0001$) for all pairs in both analyses with the matrix diagonal block excluded and included. The matrix correlation values ranged from 0.72 to 0.92 and from 0.53 to 0.82 with and without the diagonal block, respectively. The matrix correlation between covariance matrices of the island populations (matrix was generated for sample of pooled island populations) and mainland population (Volos) was high and statistically significant for the matrices with the diagonal block included ($r = 0.92$; $P < 0.0001$) and excluded ($r = 0.84$; $P < 0.0001$).

The PCA performed on residuals from the multivariate regressions extracted features of wing shape variation and showed that most variation was concentrated in a few dimensions. We displayed the features of variation associated with PC1 for each of the analysed populations (Fig. 3), accounting for 26% in Naxos, 36% in Lesbos, 42% in Chios, and 36% in Volos (mainland). In all populations, displacements of landmarks located at the wing base (1, 15, and 16) along PC1 were similar in direction and magnitude (Fig. 3).

Since shape changes at the basal part of the wing were related to FA, we compared the pattern of landmark displacements with those related to sexual dimorphism and inter-population variation within and among the analysed populations, respectively. The patterns of shape changes relating to sexual dimorphism and inter-population differentiation were obtained using data from Milankov *et al.* (unpublished manuscript). Figure 3 shows that in contrast to FA shape pattern, sexual dimorphism results from a shift of landmarks located at the posterior (Lesbos, Naxos, Volos), anterior-distal (Lesbos, Volos), and anterior-proximal (Naxos) parts and a central part of the anterior compartment (Chios, Naxos, Volos) of the wing. The pattern of shape variation among populations was a result of landmark displacements throughout the wing in males, but females were mainly differentiated by landmark shifts of the posterior compartment and both basal and apical parts of the wings.

DISCUSSION

Amounts of fluctuating asymmetry

Our results show highly significant FA, DA, and differences among individuals in wing size and shape in all studied samples. The procedure for estimating FA implemented here automatically provided correction for the presence of directional asymmetry (Klingenberg and Monteiro, 2005). For wing shape, we obtained contrasting results depending on the measure of wing shape FA used, Procrustes distance or Mahalanobis distance. The inconsistency in the results is probably due to the fact that Procrustes distance and Mahalanobis distance quantify shape variation differently (see Klingenberg and Monteiro, 2005, and references therein). Procrustes distance is a measure of the absolute magnitude of the shape deviation, whereas Mahalanobis distance provides an indication of how unusual an individual (or group) is relative to the others in the sample (in larger samples) (Klingenberg and Monteiro, 2005). There are two possible reasons for this disagreement. First, the unequal sample sizes as well as smaller sample size of the Naxos population (25 individuals) when compared with the other populations may result in a discrepancy in the results using two distances. Second, calculation of Mahalanobis distance involves steps that account for the direction of shape variation, thus if samples differ in the main direction of variation, then contrasts between Procrustes and Mahalanobis distances are expected. However, detailed analyses are needed to assess directionality of shape in multivariate space (Klingenberg and Monteiro, 2005). Similar to shape FA, the three island populations did not differ significantly in terms of size FA either. Therefore, it is likely that covariation of shape asymmetry in wings of Naxos flies is different from the other islands. However, what the proximal cause is of such observations is beyond the scope of this study.

Furthermore, by comparing shape and size FA between island and mainland populations, we discovered that the contribution of shape FA to the total shape variation was similar in the island (9.4%) and mainland populations (8.1%). Indeed, in the same manner as in the among-island comparison, the inclusion of the Naxos population in the overall island

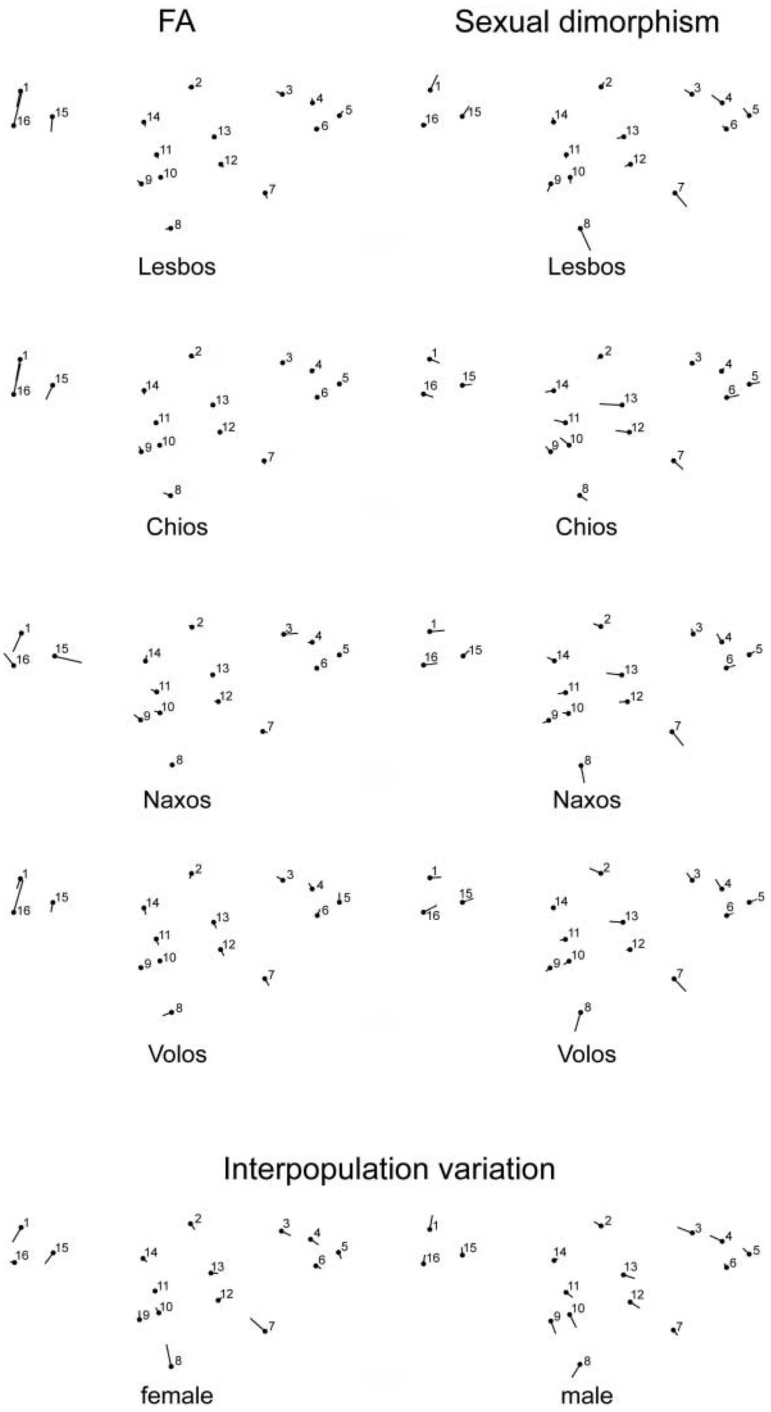

Fig. 3. PCA of variation in landmark positions for fluctuating asymmetry, sexual dimorphism, and inter-population variability in populations of *M. albifrons*. The figure shows the PC coefficients of each landmark in the x and y direction by a line originating at the mean location of the landmark (dots).

sample led the differences among island and mainland populations in shape FA to be significant, but non-significant when Naxos was omitted. In contrast to no difference in the groups' variances, the permutation test revealed significant differences between mean shape asymmetry configurations of island populations and the mainland population. In terms of size FA, and in contrast to a lack of among-island differences, size FA differed significantly when islands were compared with the mainland population of Volos. In fact, the contribution of FA compared with other sources of variation was twice that in Volos (0.67%) than in the island populations (0.33%). Therefore, our results suggest that during their development, both individuals on the mainland and on the islands may have been exposed to a variety of intrinsic (genetic) and extrinsic (environmental) stressors that could have influenced the level of developmental stability. There is a large body of evidence on the correlation between FA and genetic (e.g. inbreeding, increased homozygosity, hybridization, insecticide resistance) and environmental (e.g. pollution, food level, parasites, developmental temperature) stress (Palmer and Strobeck, 1986; Mpho *et al.*, 2002; Alibert and Auffray, 2003; Breuker *et al.*, 2006; Rego *et al.*, 2006; Carter *et al.*, 2009). However, our results are opposite to what we hypothesized. Namely, it was expected that due to the complex geological history of the Aegean, archipelago populations would experience developmental disturbance as a result of founder events, specific selection regimes, and stochastic effects coupled with restricted gene flow (Frankham, 1997; Juste *et al.*, 2001; Comes *et al.*, 2008). In fact, a significant and consistent level of FA across the analysed populations of *M. albifrons* is likely not affected by small sample size, lack of gene flow or malnutrition, since populations are numerous and relatively abundant (V. Milankov, personal communication), and a reduction of larval host plants has not been reported. Furthermore, a previous study of genetic and phenotypic diversity based on nuclear allozyme loci, COI mtDNA haplotypes, and wing size and shape revealed high genetic and phenotypic diversity and uniqueness of genetic variation at the spatial and temporal scale in *M. albifrons* (V. Milankov *et al.*, unpublished manuscript). In addition, spatial population structure did not follow a geographical gradient (isolation by distance), suggesting an important role of gene flow on spatial pattern of genetic diversity in populations from Chios and Lesbos islands (V. Milankov *et al.*, unpublished manuscript). Accordingly, the high genetic diversity parameters found in these populations imply a high capacity of the populations to respond to potential disturbances and that genetic erosion is not likely to have caused FA in *M. albifrons*.

In the absence of stressors, the question is, what causes the high level of FA? Simons and Johnston (1997) argue that increased FA does not necessarily mean low 'quality' of individuals (low fitness), but rather that selection maintains developmental instability acting directly on a character, or indirectly through the selection on a correlated character favouring high phenotypic variance. Accordingly, individuals with highly unstable and thus more variable traits would be at a selective advantage in new environments, or even asymmetry could be insignificant to survival of individuals (Simons and Johnston, 1997; Juste *et al.*, 2001).

The lack of clear differences in the level of FA among populations in our study indicates that developmental stability of wing traits is disturbed in a similar way and extent. The uniform level of shape FA and different level of size FA among island and mainland populations could be due to different genetic constraints of these two wing traits (Bitner-Mathé and Klaczko, 1999; Carreira *et al.*, 2011). That is, due to its low heritability (Bitner-Mathé and Klaczko, 1999; Moraes *et al.*, 2004), wing size is more sensitive to small variation in the environmental context than wing shape (Gilchrist and Partridge, 2001; Debat *et al.*, 2003; Breuker *et al.*, 2006). For instance, Trotta *et al.* (2005) reported that a significant increase in *Drosophila* wing size FA is associated with stressful environments. Thus, although ecologically the islands are fairly homogeneous (Comes *et al.*, 2008), the significant differences detected in wing size FA indicate possible slight differences in environmental pressures faced by island and mainland populations of *M. albifrons*. Similar to our results, no significant differences in the level of FA were found among conspecific populations or closely related species of other organisms (e.g. Hosken *et al.*, 2000; Carreira *et al.*, 2008; Sharma *et al.*, 2011). Thus, the variation of FA level is taxon, trait, and stress specific (Palmer and Strobeck, 1986; Hosken *et al.*, 2000).

Pattern of FA shape variation is not concordant with shape variation caused by sexual dimorphism and inter-population variation

In addition to non-significant differences in the amount of shape FA, the pattern of FA shape variation measured by matrix correlation and visualized using principal components analysis (PCA) on the asymmetric component of shape variation also suggested similarity among the analysed populations. Matrix correlation calculated with (full variance–covariance matrix) and without (matrix constituted of covariance alone) the diagonal blocks of the covariance matrices revealed that patterns were significant and more strongly correlated when both variance and covariance of FA were analysed. This suggests that variance of FA may be more constrained compared with joint displacements of landmarks across samples. Analysis of variation and covariation among landmarks revealed quite uniform patterns of landmark displacements in populations. We found a significant contribution of particular landmarks located in the proximal part of the wing (landmarks 1, 15, 16) in overall shape variability in all four populations. Thus, consistent FA shape pattern and localization of landmark displacements at the proximal part of the wing suggests that variation caused by perturbation during development was not transmitted throughout the entire wing. Such a discrepancy between proximal and distal parts of the wing has previously been discussed in the light of developmental processes involved in morphogenesis (Moreteau *et al.*, 1998; Debat *et al.*, 2003). Therefore, the present data suggest that different parts of the wing possess different evolutionary flexibility (plasticity), which might be caused by differences in their buffering capacity.

Furthermore, better insight into the pattern of wing shape variation has been provided by comparing the pattern of landmarks shift reflecting FA, sexual dimorphism, and inter-population variation. This is of great importance, since patterns of wing shape variation are governed by different processes and mechanisms, including developmental noise [FA (Klingenberg, 2003)], fecundity selection and sexual selection (sexual dimorphism), as well as synergistic interaction of natural selection, gene flow, and history of populations (inter-population variation). We found that landmark displacements of different parts of the wing vary, which might be influenced by different evolutionary mechanisms. Contrary to the anterior-proximal part of the wing where FA variation is localized, differences between the sexes are reflected throughout the entire wing, mostly at the distal part of both the anterior and posterior compartments. Similarly, shape variation reflecting differences among populations are roughly distributed across the wing. In general, patterns of shape variation suggest that different parts of the wing are from different ontogenic backgrounds and under different selective pressures (e.g. Pezzoli *et al.*, 1997; Debat *et al.*, 2003).

Associations of size and shape variation

We found that shape FA and centroid size FA were not correlated in any of the four populations, meaning that an increase in size asymmetry was not accompanied by more asymmetric shape. The weak association between size and shape variation was concordant across populations when both measures of shape variation (Procrustes and Mahalanobis distances) were used. To evaluate whether a lack of correlation of size and shape asymmetry is due to a direct relationship between size and shape, we tested allometry and found a significant allometric effect in populations from both the islands and mainland locality. A lack of significant correlation of FA of size and shape suggested a minimal or no developmental connection, which means that size and shape were under different developmental constraints. Wing size and shape are considered to be traits under independent genetic control and differ in heritability (Bitner-Mathé and Klaczko, 1999; Carreira *et al.*, 2011). Since wing size is considered to be a low heritable trait – compared with wing shape (Bitner-Mathé and Klaczko, 1999; Moraes *et al.*, 2004) – environmental perturbations are more likely to be connected with developmental processes underlying wing size than those responsible for wing shape, probably leading to a lack of correlation in FA of both traits. Indeed, Klingenberg and colleagues (2001) considered that correlation between asymmetries resulted from the same perturbations on the two linked traits. Contrary to the substantial allometry of FA in this study (7–14%), a significant correlation between size FA and shape FA, and relatively minor allometry (4.61%) were reported for *Drosophila melanogaster*, suggesting that a common genetic control of developmental variation of size and shape rather than allometry could explain agreement in amounts of size and shape FA (Breuker *et al.*, 2006).

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