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Genetic structure and phenotypic diversity of two northern populations of *Cheilosia* aff. *longula* (Diptera: Syrphidae) has implications for evolution and conservation

VESNA MILANKOV[1], LJUBINKA FRANCUSKI[1], JASMINA LUDOŠKI[1], GUNILLA STÅHLS[2] and ANTE VUJIĆ[1]

[1]Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Trg Dositeja Obradovi ca 2, 21000 Novi Sad, Serbia; e-mail: vesna.milankov@dbe.uns.ac.rs

[2]Finnish Museum of Natural History, PO Box 17, FIN-00014 University of Helsinki, Finland; e-mail: gunilla.stahls@helsinki.fi

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Abstract. The genetic structure and phenotypic diversity of two populations of *Cheilosia* aff. *longula* (Diptera: Syrphidae) in Lapland, Finland, were examined using DNA sequencing, protein electrophoresis, and geometric morphometrics. The morphological identification of the species were verified using partial sequences of mitochondrial cytochrome *c* oxidase subunit I (COI mtDNA), and the nuclear ribosomal internal transcribed region 2 (ITS2 rDNA), and comparing the corresponding sequences of *Cheilosia* aff. *longula* and the closely related *C. longula.* Two and three haplotypes of the genes COI mtDNA and ITS2 rDNA were identified in the two populations. Analysis of 12 isozyme loci showed an extremely low genetic variability in the populations originating from Utsjoki and Kevo. Discriminant analysis combined with canonical variate analysis revealed inter-population divergence in wing shape. Variation among genetically diverse individuals, both within- and among studied populations was studied, and directional (DA) and fluctuating asymmetry (FA) estimated using landmarks in the framework of geometric morphometrics. It is likely that the documented DA and FA asymmetry in both wing shape and size reflects the developmental instability of the individuals studied. By using Procrustes ANOVA the locations of particular landmarks responsible for the variation in shape were determined. The decomposition of the components of variance accorded to each landmark showed that the landmarks differed in the percentage of variation they accounted for (DA, FA and variation among individuals). In the discussion the implications of the reduced genetic diversity and asymmetry in wing traits for conservation is considered.

INTRODUCTION

Studies on species from Northern Europe that experienced a range of climatic changes during their history are of a special interest for studies of systematics and evolutionary and conservation biology. Until approximately 15000 years BP the whole of Northern Europe was covered with ice and both terrestrial and aquatic species experienced a hostile environment (Hewitt, 2000, 2004). The genetic consequences of repeated cycles of decreases and expansions of species' ranges, colonization and recolonization, is well documented for Fennoscandian biota (e.g. Vainio & Väinölä, 2003; Audzijonyte & Väinölä, 2006). Since genetic diversity is related to fitness and evolutionary capacity of populations (Hansson & Westerberg, 2002; Frankham, 2003; Grueber et al., 2008; Chapman et al., 2009), data on the genetic and phenotypic diversity of a Northern Boreal taxon of the family of Syrphidae (Insecta: Diptera) will provide information about the ability of the populations studied to respond to environmental changes. Hence, understanding the potential effects of selection and the possibilities for evolutionary changes has significant implications from both a fundamental and applied point of view. Therefore, this study of two northern populations of a taxon recently identified within the morphologically defined *Cheilosia longula* (Zetterstedt, 1838) species was undertaken. Molecular data indicates that *C. longula* and taxa close to *C. longula* belong to a monophyletic group, named the "*longula*" group (Claussen & Ståhls, 2007). This group comprise the species *Cheilosia longula*, *C. soror* (Zetterstedt, 1843), *C. scutellata* (Fallén, 1817), *C. flavissima* Becker, 1894, and *C. thessala* Claussen & Ståhls 2007 (Claussen & Ståhls, 2007). Based on the consistent haplotype difference at the 3' end of COI mtDNA (with uncorrected pairwise divergence of 2.88% from *C. longula*) and on distinguishing morphological characters, a new taxon provisionally named *C.* aff. *longula* was recognized (but not taxonomically formalized) within the *C. longula* group (Claussen & Ståhls, 2007). In this study molecular (DNA sequencing and protein electrophoresis) and morphometric data were used to determine the genetic and phenotypic intra- and inter-population diversity of two populations of *C.* aff. *longula* in Lapland, Finland. Two questions are addressed.

Firstly, do these populations differ both from a molecular and morphological point of view? To quantify intra- and inter-population phenotypic and genetic variation, the wing geometry, allozyme nuclear loci, mitochondrial cytochrome *c* oxidase subunit I (COI mtDNA), and nuclear ribosomal internal transcribed region 2 (ITS2 rDNA) were studied. Allozyme loci and DNA sequences of both COI mtDNA and ITS2 rDNA are widely used markers in population genetics, taxonomy, systematics,

and conservation biology of hoverflies (e.g. Milankov et al., 2008, 2009, 2010; Ståhls et al., 2009). In addition, landmark-based geometric morphometrics is used to quantify phenotypic variability (e.g. Hoffmann & Shirriffs, 2002; Ludoški et al., 2008; Olivieri et al., 2008; Francuski et al., 2009a, b). An investigation of wing geometry could be important assuming that wing shape is related to flight ability and thus to fitness (Kölliker-Ott et al., 2003). For example, Kölliker-Ott et al. (2003) report that both wing size and shape are adaptive, associated with differences in temperature and that size-related traits are under both directional and stabilizing selection. In addition, an adaptive cline in wing size in *Drosophila* is correlated with a polymorphism in a promoter region of the *Dca* (*Drosophila* cold acclimation) gene (McKechnie et al., 2010).

Secondly, are wing parameters of shape and size bilaterally symmetrical? Over the last three decades, three kinds of asymmetry have been subjected to genetical, development, and evolutionary studies (see review by Clarke, 1995; Van Dongen, 2006). The correlation between developmental instability and asymmetry of otherwise bilaterally symmetrical traits is well documented (e.g. Palmer & Strobeck, 1992; Clarke, 1993b, 1998b; Leamy & Klingenberg, 2005; Stige et al., 2006; Van Dongen, 2006; Lens & Eggermont, 2008). However, the applicability of directional asymmetry (DA), in which one side is consistently larger than the other, antisymmetry (AS), deviations from symmetry occur on both sides, and fluctuating asymmetry (FA), nondirectional subtle differences in bilaterally symmetrical traits (Van Valen, 1962; Palmer, 1994) are subjects of intense debate (see review by Lens et al., 2002; Leamy & Klingenberg, 2005). Only FA is viewed as a reliable estimator and epigenetic measure of developmental homeostasis (Palmer & Strobeck, 1992; Stige et al., 2006; Van Dongen et al., 2009). It is suggested that FA is influenced by internal factors, including genetics (high level of homozygosity and disruption of co-adapted gene complexes), physiological changes caused by extreme environmental conditions and/or environmental deterioration (Palmer & Strobeck, 1992; Willmore et al., 2007). However, there are inconsistencies in the relationships between FA and inbreeding and some components of fitness (see review by Lens et al., 2002), which has provoked an intense debate on whether FA can be considered as a "biomarker" for evaluating environmental and genetic stresses (Clarke, 1995; 1998a; Leamy & Klingenberg, 2005; Van Dongen, 2006). Still, the use of the FA of wings has proved to be an effective way of measuring developmental instability (e.g. Trotta et al., 2005; Van Dongen et al., 2009), which is important for predicting population persistence in the face of environmental and climatic change (Clarke, 1993b). It is therefore important to determine whether adaptive bilaterally symmetric traits are asymmetrical in natural populations. Hence, variation in bilaterally symmetrical traits that quantify the different components of asymmetry of wing traits (wing size and shape) were studied using a morphometric method. Finally, from a conservation point of view, it is of interest to know if this potential asymmetry in populations of *C.* aff. *longula* is linked to genetic heterogeneity.

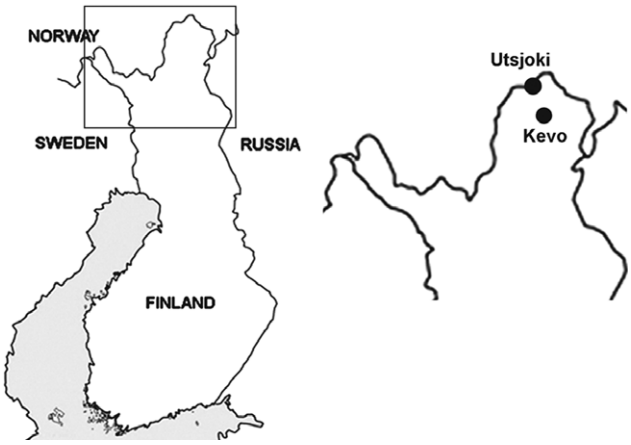

Fig. 1. Map of population sampling locations from Finnish Lapland. The collecting sites Kevo and Utsjoki are at ca 20 km distance.

MATERIAL AND METHODS

Sample collection

Claussen & Ståhls' (2007) COI mtDNA sequence data indicates the existence of a new taxon closely related to *Cheilosia longula* (Zetterstedt), but it is not formally designated. This species is included in recent faunal taxonomic studies along with a description of its diagnostic morphological characters, as it is abundant in Northern Scandinavia (Haarto & Kerppola, 2007; Bartsch et al., 2009). To verify the identity of specimens identified using a morphological key for separating all *longula* group taxa (see Haarto & Kerppola, 2007) in northern Europe, the 3' end of COI mtDNA sequences of *C. longula* and *C.* aff. *longula* were compared. According to the identification based on both morphology and COI haplotypes the specimens belong to *C.* aff. *longula*.

Two populations of *Cheilosia* aff. *longula*, one at Kevo (69°45.515′N, 26°59.428′E) and the other at Utsjoki (69°54.47′N, 27°1.592′E) (Lapland, Finland; Fig. 1) were sampled, and 46 and 18 individuals, respectively, collected in July 2007 (leg. Milankov V.). Hoverflies were stored at –20°C until used in the allozyme analysis. Altogether, 41 and 64 specimens of *C.* aff. *longula* were included in the allozyme and wing morphometric analyses, respectively. Wing traits and allozyme variation of the same specimens were analyzed and the legs of 17 specimens used for DNA sequencing.

DNA extraction and amplification

DNA was extracted from legs of the flies using a Nucleospin Tissue DNA extraction kit (Machery-Nagel, Düren, Germany) following the manufacturer's protocols, and then the DNA was re-suspended in 50 µl of ultra-pure water. Remains of specimens used for the geometric morphometric analysis, allozyme study, and DNA extraction are deposited at the University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology (Novi Sad, Serbia). PCR and sequencing followed the protocols described by Milankov et al. (2009).

The universally conserved primers used for amplifying and sequencing the COI and ITS2 fragments were C1-J-2183 (5'-CAA CAT TTA TTT TGA TTT TTT GG-3') (alias JERRY), TL2-N-3014 (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') (alias PAT) (Simon et al., 1994) and ITS2A (forward)

(5'-TGT GAA CTG CAG GAC ACA T-3'), ITS2B (reverse) (5'-TAT GCT TAA ATT CAG GGG GT-3') (Beebe & Saul, 1995), respectively. The sequences were edited for base-calling errors and assembled using Sequence Navigator™ (version 1.01) (Applied Biosystems, Foster City, CA, USA).

Allozyme analysis

The allozyme variation in 10 enzyme systems of 41 specimens was determined. The following allozyme loci were analysed by vertical polyacrylamide gel electrophoresis (PAGE): a spartate amino transferase (2.6.1.1. AAT; *Aat*), esterase (E.C. 3.1.1.? EST; two loci: *Est-2, Est-4*), fumarate hydratase (4.2.1.2. FUM; *Fum*), glucosephosphate isomerase (5.3.1.9. GPI; *Gpi*), glycerol 3-phosphate dehydrogenase (1.1.1.8. GPD; *Gpd-2*), b-hydroxy acid dehydrogenase (1.1.1.30. HAD; *Had*), hexokinase (2.7.1.1. HK; *Hk-2*), malate dehydrogenase (1.1.1.37. MDH; two loci: *Mdh-1, Mdh-2*), malic enzyme (1.1.1.40. ME; *Me*), and phosphoglucomutase (2.7.5.1. PGM; *Pgm*). Electrophoresis followed the general method of Munstermann (1979). The enzymes were analysed using two buffer systems. The Tris-Boric-EDTA buffer system (pH 8.9) was used to assay EST, FUM, GPI, HK, ME, and PGM and Tris-Citric buffer system (pH 7.1) for the analysis of AAT, HAD, GPD, and MDH. Details of buffer systems and staining procedures are given in Munstermann (1979) (EST, FUM, GPD, GPI, HK, HAD, MDH, ME, PGM) and Pasteur et al. (1988) (AAT). Duration of electrophoretic run at 90 mA (135–220 V) was 3–4 hr. Enzymes were extracted from thorax and head tissues in Tris-HCl 0.5 M pH 7.1; homogenates were centrifuged at 13 000 rpm for 3 min at 6°C.

Wing geometry

Geometric morphometric analysis was done on 46 (18♂, 28♀) and 18 (9♂, 9♀) specimens of *C.* aff. *longula* from Kevo and Utsjoki, respectively. The right and left wings of these flies were mounted in Hoyer's medium between microscope slides. A Leica DFC320 digital camera connected to a Leica MZ12.5 stereomicroscope was used to take pictures of the wings. The positions of 16 landmarks at vein intersections or terminations were assigned using TpsDig 1.40 and expressed as *x*, *y* coordinates in Cartesian space (Rohlf, 2004) (Fig. 2).

Geometric morphometrics provides a basis for understanding the variation in wing shape. It provides size-independent measures of shape, allowing unambiguous separation of size and shape. Variation in wing size was examined using centroid size (the square root of the sum of squared distance between each landmark and the wing centroid), which is an isometric estimator of size. One-way analysis of variance (ANOVA) was used to test differences in centroid size among populations and sexes.

For the analysis of wing shape the landmarks on each specimen were first aligned using a Generalized Procrustean Analysis procedure to remove the non-shape effects of translation, rotation, and scale (Rohlf & Slice, 1990) and then a thin-plate spline analysis was done. The resulting matrix (*w*; "weight matrix" of Rohlf et al., 1996) was used for a discriminant analysis combined with canonical variate analysis (CVA) to examine a classification matrix and the pattern of within-species/populations variation in total shape space. To visualize the morphological variation obtained by CVA, individual canonical scores for each root were regressed against the *x* and *y* coordinates of the original landmark positions (Rohlf et al., 1996). Differences in wing shape and size were analyzed by comparing the phenotypic traits of females and males separately.

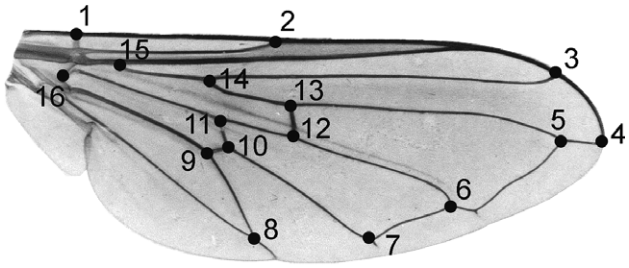

Fig. 2. The locations of 16 landmarks on wing of the *C.* aff. *longula* species selected for geometric morphometric analysis.

Procrustean superimpositions, calculation of the centroid size and *w* matrix were performed using TpsRelw 1.44 (Rohlf, 2006), multiple regressions and visualization of deformation grids were computed using TpsRegr 1.31 (Rohlf, 2005). All morphometric software of the Tps series are freely distributed at http://life.bio.sunysb.edu/morph/. ANOVA and CVA were done using Statistica for Windows (version 8.0).

Genetic analysis

Genotype and allelic frequencies were calculated directly from the observed banding patterns based on the genetic interpretation of zymograms. Calculated parameters of population genetic structure were corrected using Leven's (1949) formula for small samples. Genetic polymorphism in each population was measured using the mean number of alleles per locus (*A*), observed (H_o) and expected (H_e) heterozygosities. Allele frequencies and deviations from the Hardy-Weinberg equilibrium were calculated using BIOSYS-2 computer programme (Swofford & Selander, 1989). Assessment of genetic differentiation between populations was determined using Nei's (1978) genetic identity coefficient (*I*).

Uncorrected pairwise divergences (*p*-distances) were calculated based on the COI sequences of 17 specimens.

Asymmetry

Here, the levels of FA in multiple wing traits (size and shape) were assed within and between the two populations of *C.* aff. *longula*. For this purpose, wing asymmetry, based on the statistically significant right-left differences in wing size and shape, was studied. Asymmetry was measured using both the left and right wings of 44 (18♂ and 26♀) and 12 (6♂ and 6♀) specimens from Kevo and Utsjoki, respectively.

Asymmetry in wing shape and size was estimated using geometric morphometrics. Two-way ANOVA on repeated measurements (Palmer, 1994) was used to assess asymmetry in centroid size. In the model implemented herein, "Individuals" (I) refers to a random factor that assesses variation among individuals, "Sides" (S) is a fixed factor that assesses directional asymmetry (DA), the Individuals × Sides interaction assesses fluctuating asymmetry (FA), and the error assesses variation in the replicate measurements (Leamy, 1984; Palmer, 1994). The Procrustes analysis was used to compute best-fitting superimpositions of configurations of landmarks to the left and right sides of a single specimen. Asymmetry in wing shape was tested using Procrustes ANOVA (Klingenberg & McIntyre, 1998) with the landmark coordinates of the Procrustes-aligned configurations (all three replicates of each wing) as the data. The MS (mean square; obtained using regular ANOVAs and Procrustes ANOVAs) related to the individual effect and to the individual × side interaction were used as an estimator of the amount of individual variation and FA for wing size and shape. To examine the effects of particular landmarks of shape changes, separate $ANOVA_s$ on landmark coordinates were calculated and then the

TABLE 1. Allelic frequency at variable loci and estimates of genetic structure of populations of *C.* aff. *longula*.

Population		Utsjoki	Kevo
Loci/allele			
Me	*a*	–	0.192
	b	0.643	0.538
	c	0.357	0.269
Pgm	*a*	–	0.058
	b	1.000	0.904
	c	–	0.038
n (SE)		7.1 (1.3)	20.8 (1.7)
A (SE)		1.1 (0.1)	1.3 (0.2)
$P_{(0.95)}$		8.3	16.7
H_o (SE)		0.000	0.016 (0.016)
H_e (SE)		0.038 (0.038)	0.065 (0.051)

n – mean sample size per locus; SE – standard error; *A* – mean number of alleles per locus; *P* – frequency of polymorphic loci based on the criterion of 0.95; H_o – average frequency of observed heterozygosity; H_e – average frequency of expected heterozygosity.

x- and *y*-mean squares of each landmark were summed (Klingenberg & McIntyre, 1998; Badyaev & Foresman, 2000). The multivariate patterns of landmark covariation were studied using principal component analysis (PCA) following Klingenberg & McIntyre (1998) and Klingenberg & Zaklan (2000). To study the developmental basis of FA and variation among individuals covariance matrices were computed and compared with multivariate patterns of landmark covariation corresponding to within-individual variability and variation among individuals. Analysis of asymmetry of wing size and shape was done using Statistica for Windows (version 8.0).

RESULTS

Mitochondrial COI and nuclear DNA diversity

A 764 bp fragment of the 3' end of COI mtDNA gene, corresponding to nucleotide positions 2207 to 2970 in the *Drosophila yakuba* sequence (Clary & Wolstenholme, 1985), was obtained from nine and eight specimens of the populations sampled at Kevo and Utsjoki, respectively. Additionally, a fragment of nuclear ITS2 rDNA was obtained from seven and eight specimens from Kevo and Utsjoki, respectively. All sequences are deposited in GenBank and the accession numbers of the analysed specimens are FJ158617–FJ158648.

Sequencing of the 3' end of COI revealed three haplotypes, two of which were unique to these populations. A private haplotype of one specimen from Utsjoki (acc. no. FJ158624) was different in one and two nucleotides from the common and a private haplotype recorded in the Kevo population, respectively. Another unique haplotype observed in a specimen from the Kevo population (acc. no. FJ158619) also differed from the common haplotype at one nucleotide site. Thus, uncorrected pairwise divergences (*p*-distances in %) estimated among the common haplotype and private haplotypes, and between the two private haplotypes of the populations were 0.13% and 0.29%, respectively.

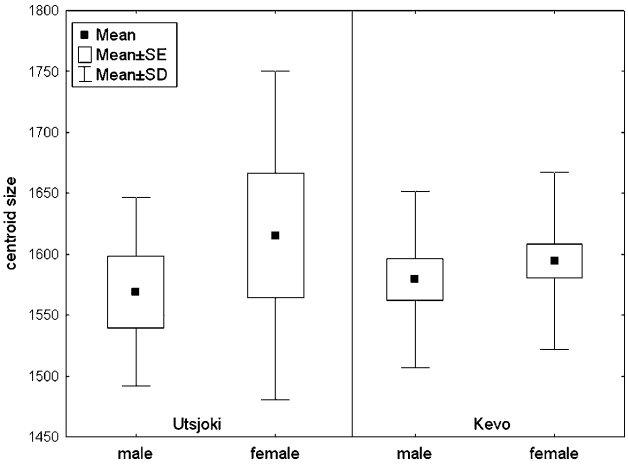

Fig. 3. Boxplot of centroid size of populations of the *C.* aff. *longula* species with the mean, standard error, and standard deviation illustrating between sex and interpopulation variation in right wing size. Non-significant differences were calculated between the populations from Utsjoki and Kevo.

The 14 sequences of ITS2 rDNA were identical, and only one specimen from Utsjoki was different (acc. no. FJ158618). However, this specimen possessed a common haplotype of COI mtDNA.

Allozyme variability

Data from 15 and 26 individuals from Utsjoki and Kevo, respectively, were obtained. Of the 12 isozyme loci analyzed, 10 were monomorphic for the same alleles in both populations. The *Me* locus was polymorphic in both populations and *Pgm* was variable in the population at Kevo (Table 1). The spatial distribution of the two alleles (Me^b, Me^c) in both populations and one (Me^a) in the Kevo population, indicate genetic divergence among conspecific populations. Only at the *Pgm* locus in the population from Kevo were two heterozygotes, $Pgm^{a/b}$ (frequency 0.115) and $Pgm^{b/c}$ (frequency 0.077), detected. Thus, within the population from Kevo there are two unique alleles at *Pgm* (Pgm^a, Pgm^c). The average number of alleles per locus (*A*) was 1.1 and 1.3 for the populations at Utsjoki and Kevo, respectively (Table 1).

Chi-squared tests for deviations of observed genotypes from expected revealed statistically significant departure from Hardy-Weinberg equilibrium at the *Me* locus in both populations, but not at *Pgm* for the population at Kevo. Consequently, the observed heterozygosity (H_o) was generally lower than expected heterozygosity (H_e), and the genotype fixation index, F_{IS}, indicated excess homozygosity ($F_{IS} > 0$) at all analysed loci in the populations, except *Pgm* in the population at Kevo. The value of genetic identity between populations was $I = 0.998$.

Phenotypic variability

Differences between the populations and sexes of *C.* aff. *longula* were examined using both the right and left wings. ANOVA on centroid size of right (male: $F_{(1,23)} = 0.102$, $P = 0.75$; female: $F_{(1,33)} = 0.316$, $P = 0.58$) and left (male: $F_{(1,24)} = 0.138$, $P = 0.71$; female: $F_{(1,32)} = 0.098$, P

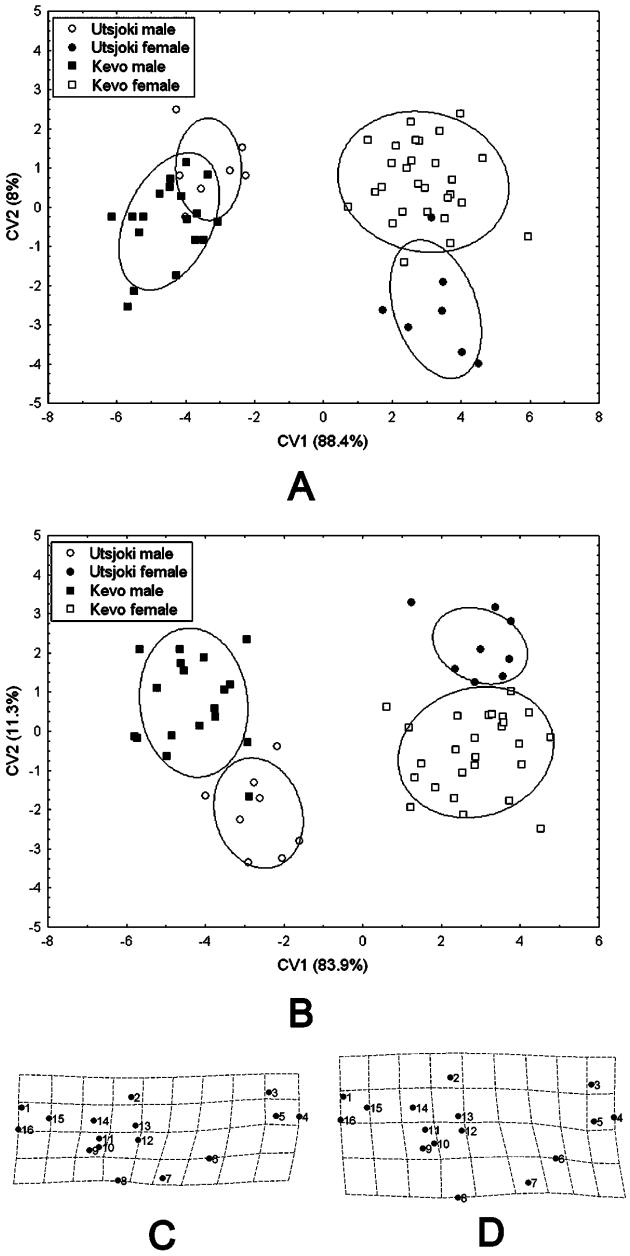

Fig. 4. Scatterplot of individual scores from the CVA showing shape differentiation of right (A) and left (B) wing between populations and between sexes of *C.* aff. *longula*. The amount of variation explained by each axis is in parentheses. Thin-plate spline reconstruction representing negative (C) and positive (D) deformations of mean shape between genders along the CV1 axis. Deformation grids are exaggerated × 3. Numbers in the deformation grids refer to landmarks shown in Fig. 2.

= 0.76) wings revealed no significant interpopulation differences (Fig. 3).

Discriminant analysis, combined with canonical variate analysis (CVA) of the *w* matrix of the right wing of both sexes, revealed that 88.9% of both sexes at Utsjoki, and 94.7% of males and 100% of females at Kevo were correctly classified (overall classification success was 95%; Wilks' $\Lambda = 0.02$, $F_{(84,87)} = 2.79$, $P < 0.001$), which indicates that wing shape can be used to discriminate between populations (Fig. 4a). Similarly, the second canonical axis discrimination between populations based on the shape of the left wing with an overall classification success of 96.6% (Utsjoki: 100% male and 85.7% female, Kevo: 94.7% male and 100% female; Wilks' $\Lambda = 0.02$, $F_{(84,87)} = 2.99$, $P < 0.001$) (Fig. 4b).

Sexual size dimorphism based on both the right wing centroid size (Utsjoki: $F_{(1,12)} = 0.62$, $P = 0.45$; Kevo: $F_{(1,44)} = 0.46$, $P = 0.50$) (Fig. 3) and left wing centroid size (Utsjoki: $F_{(1,14)} = 0.17$, $P = 0.69$; Kevo: $F_{(1,42)} = 1.03$, $P = 0.32$) was not detected in either population. However, there was a highly significant sexual dimorphism in shape when both the right (Wilks' $\Lambda = 0.004$, $F_{(149,158)} = 2.24$, $P < 0.001$) and left (Wilks' $\Lambda = 0.006$, $F_{(149,158)} = 2.08$, $P < 0.001$) wings were analysed separately (Fig. 4a, b). For both wings the CVA discriminated between the sexes along the first canonical axis. The thin-plate spline visualizations showed that most of the differences in the shape between the sexes were associated with differences in relative positions of landmarks 2 and 4–8, which reflect wing width and length (Fig. 4c, d).

Asymmetry

ANOVA of the FA of both wing shape and size for both sexes from the Utsjoki and Kevo populations revealed that the main effect of Individuals and the Individual × Side interaction were highly significant. FA was also estimated based on the observed significant Individual × Side interaction. For both traits, ANOVA indicated significant differences among individuals and therefore significant FA and DA (Tables 2 and 3).

A more differentiated pattern emerged when variance components from the Procrustes ANOVA of wing shape apportioned by landmarks in both sexes were analysed separately. For DA (Side effect) the largest share of the variation is located at the landmarks defining outer margin (landmark 7 in males from both populations and 1 in females from Utsjoki) and the position of the crossveins (10, 11, 15, and 16 in females from Kevo). Thus, in contrast to landmark 7 in males, which has a proportional share in DA, there was a disproportionate variation of different landmarks in DA in females from these populations. The largest left-right differences within individuals (FA; S × I effect) were found at the basal landmark 16 in both sexes from both populations and at the anterior outer wing margin in males (1 and 2) and females (1) from Utsjoki. Unlike the population from Utsjoki, the amount of FA variation was largest at landmark 7 located in the posterior part of the wing. In contrast to landmark 16, which had large amount of shape variation in FA in both sexes from Kevo and Utsjoki, other landmarks had disproportionate amounts of variation in these populations. Finally, the effects of individual variation (I) were distributed mainly among landmarks defining the outer margin of the posterior part of the wing (7 and 8 in both sexes from Kevo, and 7 and 6, 8 in females and males from Utsjoki, respectively). Landmarks defining the anterior part of the wing (2, 4 in males and 2 in females) had the largest individual effect (variation among individuals) in specimens from Kevo and inner landmarks 12 and 13 the largest effect on variation in shape in both sexes from

TABLE 2. Two-way ANOVA of centroid size and Procrustes ANOVA of wing shape based on repeated measurements and used to asses directional asymmetry (sides effect) and fluctuating asymmetry (individuals × sides interaction effect) in the population of *C.* aff. *longula* from Utsjoki.

Source		Male			Female		
		df	MS	*F*	df	MS	*F*
Centroid size	Side (S)	1	97.38	34.26***	1	314.17	69.65***
	Individual (I)	5	40454.67	14233.07***	5	129472.5	28705.19***
	S × I	5	94.69	33.32***	5	108.93	24.15***
	Error	24	2.84		24	4.51	
Shape	Side (S)	28	0.000005	4.47***	28	0.000007	8.50***
	Individual (I)	140	0.000100	97.34***	140	0.000067	80.73***
	S × I	140	0.000008	7.97***	140	0.000006	7.36***
	Error	672	0.000001		672	0.000001	

*** $P < 0.001$

Utsjoki and males from Kevo, respectively (results not shown).

In order to determine whether within-individual variability (FA) and variation among individuals have similar patterns of landmark covariation the patterns in landmark positions were compared. PCA of the matrices of variance and covariance components for the different ANOVA effects revealed that most of the variance among individuals and most FA (individual × side interaction) variance in both sexes in the two populations was concentrated in the first few dimensions, with slightly more for individual variation than for FA for both females and males from Kevo. Unlike the population from Kevo, variation accounted for by FA was slightly more than variation among individuals in both sexes originating from Utsjoki. Similar principal components (PCs) for both within-individual variation (FA) and variation among individuals differ from those attributed to measurement error in both populations (Figs 5 and 6), which implies that similar developmental processes control fluctuating asymmetry and individual variation. In contrast to the amount of FA in shape, individual variation varies within samples, especially the female sample from Utsjoki (Fig. 7).

DISCUSSION

Genetic and phenotypic diversity

Regarding the question whether there is genetic and phenotypic differentiation among northern hoverflies our results reveal inter-population divergence between populations of *C.* aff. *longula*. A comparison of the genetic diversity values of populations at Kevo and Utsjoki indicated differences in the spatial distributions of alleles at the *Me* and *Pgm* loci. Private alleles at the *Pgm* and *Me* loci in the population from Kevo and phenotypic divergence in wing shape (contrary to wing size) are good indicators of population structuring. Indeed, each of the populations studied has a distinct wing shape, suggesting clear inter-population phenotypic divergence. This finding is important since differences in wing shape and size are constrained genetically and regarded as adaptive. Although the genetic determination of morphometric characters are still relatively poorly known, Birdsall et al. (2000) suggest that genes that regulate wing shape are more tightly connected than those that determine wing size and the former are probably associated with determination of wing veins. Moreover, McKechnie et al. (2010) reports an adaptive variation in wing size in populations of *Drosophila melanogaster* across climatic gradients and an association between wing size and *Dca* alleles. Genetic basis of wing size polymorphism is additionally sup-

TABLE 3. Two-way ANOVA of centroid size and Procrustes ANOVA of wing shape based on repeated measurements and used to asses directional asymmetry (sides effect) and fluctuating asymmetry (individuals × sides interaction effect) in the population of *C.* aff. *longula* from Kevo.

Source		Male			Female		
		df	MS	*F*	df	MS	*F*
Centroid size	Side (S)	1	418.50	236.42***	1	56.20	19.76***
	Individual (I)	17	32063.66	18113.15***	25	26797.50	9423.41***
	S × I	17	86.36	84.79***	25	54.08	19.02***
	Error	72	1.77		104	2.84	
Shape							
	Side (S)	28	0.000007	6.92***	28	0.000007	7.88***
	Individual (I)	476	0.000113	108.41***	700	0.000112	123.86***
	S × I	476	0.000007	6.87***	700	0.000007	7.31***
	Error	2016	0.000001		2912	0.000001	

*** $P < 0.001$

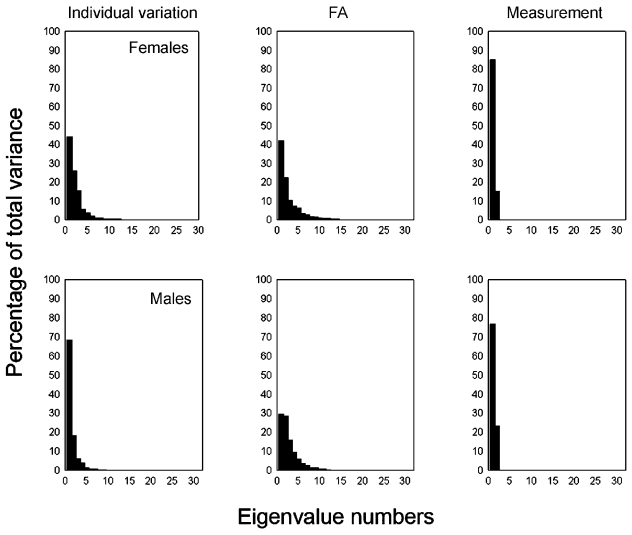

Fig. 5. Percentage of total shape variation taken up by the principal components of the matrices of variance and covariance components for individual variation, fluctuating asymmetry, and measurement error in population from Kevo.

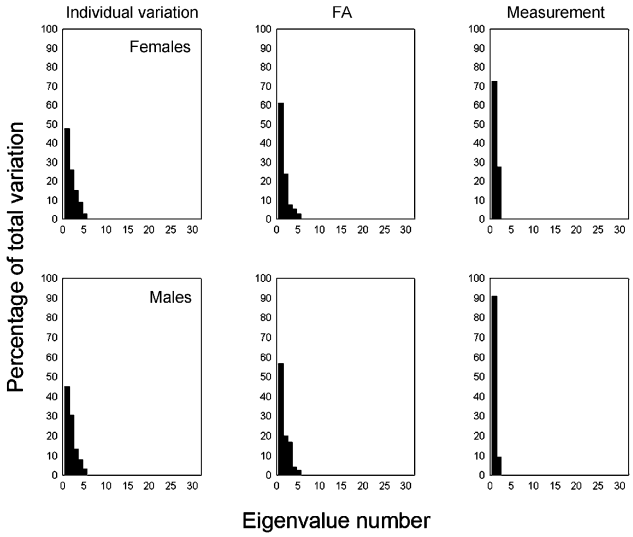

Fig. 6. Percentage of total shape variation taken up by the principal components of the matrices of variance and covariance components for individual variation, fluctuating asymmetry, and measurement error in population from Utsjoki.

ported by the linkage between the *Dca* gene and genes located within the *In(3R)P* inversion (Kennington et al., 2007). Thus, it is likely this cline in size is a result of thermal selection acting on flight (Blanckenhorn & Demont, 2004). Hence, a lack of phenotypic divergence in wing size documented for *C.* aff. *longula* probably reflects similar thermal regimes at the localities of the populations studied.

In addition, slight variation in both mitochondrial (a common COI haplotype is dominant in both populations) and nuclear data (monomorphic allozyme genes and presence of identical ITS2 rDNA sequences in all specimens studied, except one originating from Utsjoki) was observed. This low level of intra-population genetic variation at allozyme loci is recorded in hoverfly populations in small, spatially fragmented and/or isolated populations of taxa within the genera *Melanogaster*, *Merodon*, and *Cheilosia* (Milankov et al., 2010). The low level of genetic diversity detected in this study is similar to that recorded for the northern hoverfly, *Cheilosia naruska* Haarto & Kerppola, 2007 (Milankov et al., 2010). Although there is little information on the genetic diversity of northern populations [data only for *C. naruska* (Milankov et al., 2010) and that presented in this study] compared with southern taxa, it seems evident that less genetic variation might be expected. In comparison with Southern Europe, there are fewer species and less genetic diversity in Northern Europe (Hewitt, 2000). Thus a reduced genetic diversity might be characteristic of northern populations of both hoverfly species, *C. naruska* (Milankov et al., 2010) and *C.* aff. *longula* (reported herein). The similar levels of genetic variation in northern hoverfly species suggest that they have experienced similar ecological factors and evolutionary histories.

This study revealed sexual dimorphism in wing shape but not wing size. This finding accords with expectation since sexual biased wing shape is common in Diptera (Birdsall et al., 2000; Monteiro et al., 2002; Matta & Bitner-Mathé, 2004) and some hoverflies (Ludoški et al., 2008; Francuski et al., 2009a, b; Milankov et al., 2010). Here it is documented that homologous landmarks tend to have opposite displacement in females and males. For example, landmarks 4 and 5 are in a more distal position in males than females, landmark 2 is a more anterior in males, and landmarks 6, 7, and 8 are more posterior than those in females (Fig. 4). These results are similar to those of studies on variation in wing compartments in which functional constraints determine wing shape (Gilchrist et al., 2000).

Asymmetry

The second aim, to study the left-right axis in two populations of a northern species of hoverfly involved the analysis of wing size and shape using a geometric morphometric method to quantify the left-right differences. This revealed the presence of both DA and FA in both the size and shape of a bilaterally symmetrical organ, the wings. In addition, there were no differences between sexes in the level of FA. Wing left-right asymmetry is thought to be due to the separate development of imaginal discs (Klingenberg et al., 1998) and that the same perturbations influence the developmental pathways of two traits. This asymmetry is likely to affect wing aerodynamic properties and hence impede flight performance. Asymmetry in wing shape is reported in number of insect species, for example, *Apis mellifera* (Smith et al., 1997), *Drosophila melanogaster* (Breuker et al., 2006; Klingenberg et al., 1998), *Musca domestica* and *Glossina palpalis gambiensis* (Klingenberg et al., 1998), and *Carabus solieri* (Garnier et al., 2006). Currently, there is little information on the variation between left-right wing traits in hoverflies. For instance, FA of wing size is recorded in a population of a northern species, *Cheilosia naruska* (Milankov et al., 2010) and populations on Aegean

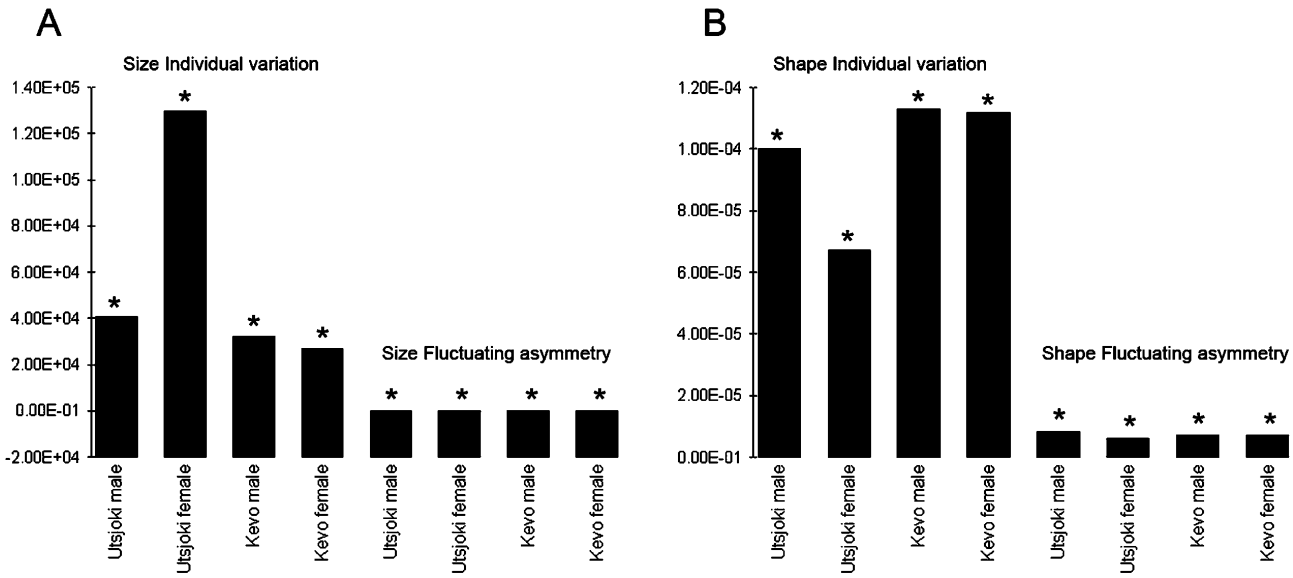

Fig. 7. Levels of Individual variation and Fluctuating asymmetry for size (A) and shape (B). Each graphic shows MS value corresponding to male and female individuals from Utsjoki and Kevo. * $P < 0.001$

islands of a southern species *Merodon albifrons* (V. Milankov, J. Ludoški, L. Francuski, G. Ståhls, A. Vujić, unpubl. data). However, unlike in the northern populations of both *Cheilosia* species (*C. naruska*, Milankov et al., 2010; *C.* aff. *longula*, reported herein) and the autumn generation of *M. albifrons*, FA of wing shape was not detected in the spring generation of *M. albifrons* (V. Milankov, J. Ludoški, L. Francuski, G. Ståhls, A. Vujić, unpubl. data). Similar relationships between individual and FA of wing shape and size in *Drosophila* are attributed to the different genetic properties of both traits (Breuker et al., 2006).

Furthermore, the variation among individuals and within-individual asymmetry (FA) between body sides were examined and the multivariate patterns of landmark covariation throughout the wing compared. There were differences in the degree of variability among landmarks in both populations when the sexes were considered separately. These findings revealed distinct patterns of landmark displacements at the different levels, which implied different amounts of variation in localized shape in the overall variability. For example, the significant components of variation (both within and among individuals) were confined to the outer margin of the wing. In addition, the PCA showed that FA and individual variation are concentrated within the first few principal components (PCs). Also contrary to the observed similar patterns in FA and individual variation, the distribution of eigen values for measurement error was markedly different for both sexes in the populations studied. Similar patterns of PCs for individual variation and FA in the *Drosophila* wing are linked to the effects of subtle perturbations during development of the wing (Klingenberg & Zaklan, 2000). In addition, concordance between individual variation and FA is thought to reflect a similar response of the developmental processes to perturbations during development, and, therefore, a lack of distinct developmental processes that generate variation within individuals (FA) (Klingenberg & McIntyre, 1998).

Conservation implications

The low genetic variation documented herein is possibly correlated with a lower evolutionary potential, which is already suggested for endangered and threatened species (e.g. Reed & Frankham, 2003; Frankham, 2005; Grueber et al., 2008). This relationship between genetic diversity and the evolutionary potential of a population is of great importance; low genetic variation influences short-term decreases in fitness through inbreeding depression and long-term lack of adaptive flexibility (e.g. David, 1998; Frankham et al., 2002; Hansson & Westerberg, 2002; Grueber et al., 2008; Malo & Coulson, 2009). Because of this, studies on heterozygosity-fitness correlations are highlighted in conservation genetics and management of threatened species (Hansson & Westerberg, 2002; Grueber et al., 2008; Chapman et al., 2009). As inbreeding depression and loss of genetic diversity increase the risk of extinction (Frankham, 2005; Grueber et al., 2008), populations of *C.* aff. *longula* with reduced genetic diversity are likely to have a low potential for adapting to a change in the environment.

Due to their functional importance and, therefore, high degree of developmental canalization (influenced by strong stabilizing selection) (Møller, 1993), wings can be used to study the ability of individuals to cope with environmental and internal stress (see review by Palmer & Strobeck, 1986). Both the FA and DA in wing shape and size recorded in both populations indicate that wing discs experience disturbance during early embryogenesis. It is likely that a shift in the developmental pathway might result from both exterior and interior factors, including nutritional stress and lack of heterozygosity. One of the possible factors influencing observed FA in *C.* aff. *longula* might be an unstable developmental environment caused by fast and unpredictable deterioration in the food

of the larvae. Recent re-examination of previously reared material of G. Ståhls (unpubl. data) revealed that *C.* aff. *longula* is only reared from *Leccinum versipelle* and *L. scabrum*, while the closely related *Cheilosia longula* is mainly reared from species of the genus *Suillus*. A study of *C. longula* reared from *Leccinum* spp. in Western Finnish Lapland showed that they are as abundant in sporophores as *Pegomya* spp. (Diptera: Anthomyiidae) (> 20 individuals/sporophore). In addition, the size of the individual flies (as measured by length of thorax) is negatively correlated with the number of flies emerging, which suggests an increase in competition with increasing numbers of larvae in individual sporophores (Ståhls et al., 1989). Therefore, the development of larvae exploiting an ephemeral food source like a fungus is subject to environmental and biotic factors affecting the larval life-cycle that are temporally and spatially highly variable and likely to affect the quality of the substrate. Likewise, stressful conditions, such as a poor or a lack of the preferred food source, are likely to affect wing development in phytophagous insects. For example, Soto et al. (2008) reveal that levels of FA in wing size in *Drosophila buzzatii* and *D. koepferae* depend on the nature of the breeding substrate.

On the other hand, the wing shape of heterozygote and homozygote individuals differ (Messier & Mitton, 1996). Our results accord with previous findings, as a correlation between heterozygosity based on variation in proteins and lower FA is documented in several studies (e.g. Palmer & Strobeck, 1986; Clarke 1993a, Hartl et al., 1995; Messier & Mitton, 1996; Smith et al., 1997; Vøllestad et al., 1999; Borrell et al., 2004; Milankov et al., 2010). It is argued that genomic heterozygosity in protein loci influences developmental stability in such way that increasing homozygosity affects the (in)ability of organisms to buffer against developmental instability (refers to the direct effect hypothesis of the multilocus heterozygosity-fitness correlations) (Møller, 1993; Kark, 2001; Hansson & Westerberg, 2002; Grueber et al., 2008). In addition, the different internal environments an individual experiences during early embryonic development determine the effect that decreased heterozygosity has on developmental instability, which is reflected in a high level of FA, is more important in ectotherms than endotherms (Vøllestad et al., 1999). In addition, presence and strengths of relationships between FA, stress and fitness depend on trait type and species (see review Lens et al., 2002). Since the genetic basis of developmental stability might be character-, population-, and taxon-specific (Clarke, 1998b; Hansson & Westerday, 2002), and affected by a variety of external (abiotic and biotic) and internal (genetic) stresses (Trotta et al., 2005), FA should be correlated between traits within individuals. Given all the factors that influence development (mentioned above), the inconsistent results of a wide range of studies on the association between heterozygosity and developmental stability in diverse taxa is not surprising (Palmer & Strobeck, 1986; Clarke et al., 1992; Clarke, 1993a; Vøllestad et al., 1999).

However, as little is known about the evolutionary implications of developmental instability in hoverflies it is hoped that the results presented herein will stimulate further research on the processes affecting the development of wings. The next step in this study will be a simultaneous analysis of genetic (heterozygosity) and environmental (nutrition) factors, and the asymmetry of multiple traits in the species studied. Finally, integrating morphometric and genetic analyses could prove a promising tool for evaluating the evolutionary potential of natural populations and understanding the evolutionary processes involved.

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REFERENCES

AUDZIJONYTE A. & VÄINÖLÄ R. 2006: Phylogeographic analyses of a circumarctic coastal and a boreal lacustrine mysid crustacean, and evidence of fast postglacial mtDNA rates. *Mol. Ecol.* **15**: 3287–3301.

BADYAEV A.V. & FORESMAN K.R. 2000: Extreme environmental change and evolution: stress – induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proc. R. Soc. Lond. (B)* **267**: 371–377.

BARTSCH H., BIENKIEWICZ E., RÅDEN A. & NASIBOV E. 2009: Nationalnyckeln till Sveriges flora och fauna. Tvåvinger: Blomflugor: Eristalinae och Microdontinae. Diptera: Syrphidae: Eristalinae & Microdontinae. ArtDatabanken, SLU Uppsala.

BEEBE N.W. & SAUL A. 1995: Discrimination of all memebers of the Anopheles punctulatus complex by polymerase chain reaction-restriction fragment length polymorphism analysis. *Am. J. Trop. Med. Hyg.* **53**: 478–481.

BIRDSALL K., ZIMMERMAN E., TEETER K. & GIBSON G. 2000: Genetic variation for the positioning of wing veins in Drosophila melanogaster. *Evol. Dev.* **2**: 16–24.

BLANCKENHORN W.U. & DEMONT M. 2004: Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum. *Integr. Comp. Biol.* **44**: 413–424.

BORRELL Y.J., PINEDA H., MCCARTHY I., VAZGUEZ E., SANCHEZ J.A. & LIZANA G.B. 2004: Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, Salmo salar L. *Heredity* **92**: 585–593.

BREUKER C.J., PATTERSON J.S. & KLINGENBERG C.P. 2006: A single basis for developmental buffering of Drosophila wing shape. *PLoS ONE* **1**: e7.

CHAPMAN J.R., NAKAGAWA S., COLTMAN D.W., SLATE J. & SHELDON B.C. 2009: A quantitative review of heterozygosity-fitness correlations in animal populations. *Mol. Ecol.* **18**: 2746–2765.

CLARKE G.M. 1993a: The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. *Genetica* **89**: 15–23.

CLARKE G.M. 1993b: Fluctuating asymmetry of invertebrate populations as biological control of environmental quality. *Environ. Pollut.* **82**: 207–211.

CLARKE G.M. 1995: Relationships between developmental stability and fitness: application for conservation biology. *Conserv. Biol.* **9**: 18–24.

CLARKE G.M. 1998a: Developmental stability and fitness: the evidence is not quite so clear. *Am. Nat.* **152**: 762–766.

CLARKE G.M. 1998b: The genetic basis of developmental stability. IV Individual and population asymmetry parameters. *Heredity* **80**: 553–561.

CLARKE G.M., OLDROYD B.P. & HUNT P. 1992: The genetic basis of developmental stability in Apis mellifera: heterozygosity versus genic balance. *Evolution* **46**: 753–762.

CLARY D. & WOLSTENHOLME D. 1985: The mitochondrial DNA molecule of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.* **22**: 252–271.

CLAUSSEN C. & STÅHLS G. 2007: A new species of Cheilosia Meigen from Thessaly / Greece, and its phylogenetic position (Diptera, Syrphidae). *Volucella* **8**: 45–61.

DAVID P. 1998: Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity* **80**: 531–537.

FRANCUSKI L., LUDOŠKI J., VUJIĆ A. & MILANKOV V. 2009a: Wing geometric morphometric inferences on species delimitation and intraspecific divergent units in the Merodon ruficornis group (Diptera, Syrphidae) from the Balkan Peninsula. *Zool. Sci.* **26**: 301–308.

FRANCUSKI L., VUJIĆ A., KOVACEVIC A., LUDOŠKI J. & MILANKOV V. 2009b: Identification of the species of the Cheilosia variabilis group (Diptera, Syrphidae) using wing geometric morphometrics with the revision of status of C. melanopa redi Vujić, 1996. *Contrib. Zool.* **78**: 129–140.

FRANKHAM R. 2003: Genetics and conservation biology. *C.R. Biologies* **326**: S22–S29.

FRANKHAM R. 2005: Genetics and extinction. *Biol. Conserv.* **126**: 131–140.

FRANKHAM R., BALLOU J.D. & BRISCOE D.A. 2002: *Introduction to Conservation Genetics.* Cambridge University Press, Cambridge, 617 pp.

GARNIER S., GIDASZEWSKI N., CHARLOT M., RASPLUS J.-Y. & ALIBERT P. 2006: Hybridization, developmental stability, and functionality of morphological traits in the ground beetle Carabus solieri (Coleoptera, Carabidae). *Biol. J. Linn. Soc.* **89**: 151–158.

GILCHRIST A.S., AZEVEDO R.B.R., PARTRIDGE L. & O'HIGGINS P. 2000: Adaptation and constraint in the evolution of Drosophila melanogaster wing shape. *Evol. Dev.* **2**: 114–124.

GRUEBER C.E., WALLIS G.P. & JAMIESON I.G. 2008: Heterozygosity-fitness correlations and their relevance to studies on inbreeding depression in threatened species. *Mol. Ecol.* **17**: 3978–3984.

HAARTO A. & KERPPOLA S. 2007: *Finnish Hoverflies and Some Species in Adjacent Countries.* Otavan Kirjapaino Oy, Keuruu, 647 pp.

HANSSON B. & WESTERBERG L. 2002: On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.* **11**: 2467–2474.

HARTL G.B., SUCHENTRUNK F., WILLING R. & PETZNEK R. 1995: Allozyme heterozygosity and fluctuating asymmetry in the brown hare (Lepus europeus): a test of the developmental homeostasis hypothesis. *Phil. Trans. R. Soc. Lond. (B)* **350**: 313–323.

HEWITT G.M. 2000: The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

HEWITT G.M. 2004: Genetic consequences of climatic oscillations in the Quaternary. *Phil. Trans. R. Soc. Lond. (B)* **359**: 183–195.

HOFFMANN A.A. & SHIRRIFFS J. 2002: Geographic variation for wing shape in Drosophila serrata. *Evolution* **56**: 1068–1073.

KARK S. 2001: Shifts in bilateral asymmetry within a distribution range: the case of the chukar partridge. *Evolution* **55**: 2088–2096.

KENNINGTON W.J., HOFFMANN A.A. & PARTRIDGE L. 2007: Mapping regions within cosmopolitan inversion In(3R)payne associated with natural variation in body size in Drosophila melanogaster. *Genetics* **177**: 549–556.

KLINGENBERG C.P. & MCINTYRE G.S. 1998: Geometric morphometrics of developmental instability: analysing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* **52**: 1363–1375.

KLINGENBERG C.P. & ZAKLAN S.D. 2000: Morphological integration between developmental compartments in the Drosophila wing. *Evolution* **54**: 1273–1285.

KLINGENBERG C.P., MCINTYRE G.S. & ZAKLAN S.D. 1998: Left-right asymmetry of wings and the evolution of body axes. *Proc. R. Soc. Lond. (B)* **265**: 1255–1259.

KÖLLIKER-OTT U.M., BLOWS M.W. & HOFFMANN A.A. 2003: Are wing size, wing shape and asymmetry related to field fitness to Trichogramma egg parasitoids? *Oikos* **100**: 563–573.

LEAMY L. 1984: Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. *Am. Nat.* **123**: 579–593.

LEAMY L.J. & KLINGENBERG C.P. 2005: The genetics and evolution of fluctuating asymmetry. *Annu. Rev. Ecol. Evol. Syst.* **36**: 1–21.

LENS L. & EGGERMONT H. 2008: Fluctuating asymmetry as a putative marker of human-induced stress in avian conservation. *Bird Conserv. Int.* **18**: S125–S143.

LENS L., VAN DONGEN S., KARK S. & MATTHYSEN E. 2002: Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biol. Rev.* **77**: 27–38.

LEVENE H. 1949: On a matching problem arising in genetics. *Ann. Math. Stat.* **20**: 91–94.

LUDOŠKI J., FRANCUSKI L., VUJIĆ A. & MILANKOV V. 2008: The Cheilosia canicularis group (Diptera: Syrphidae): species delimitation and evolutionary relationships based on wing geometric morphometrics. *Zootaxa* **1825**: 40–50.

MALO A.F. & COULSON T. 2009: Heterozygosity-fitness correlations and associative overdominance: new detection method and proof of principle in the Iberian wild boar. *Mol. Ecol.* **18**: 2741–2742.

MATTA B.P. & BITNER-MATHÉ B.C. 2004: Genetic architecture of wing morphology in Drosophila simulans and an analysis of temperature effects on genetic parameter estimates. *Heredity* **93**: 330–341.

MCKECHNIE S.W., BLACKET M.J., SONG S.V., RAKO L., CARROL X., JOHNSON T.K., JENSEN L.T., LEE S.F., WEE C.W. & HOFFMANN A.A. 2010: A clinally varying promoter polymorphism associated with adaptive variation in wing size in Drosophila. *Mol. Ecol.* **19**: 775–784.

MESSIER S. & MITTON J.B. 1996: Heterozygosity at the malate dehydrogenase locus and developmental homeostasis in Apis mellifera. *Heredity* **76**: 616–622.

MILANKOV V., STÅHLS S., STAMENKOVIĆ J. & VUJIĆ A. 2008: Genetic diversity of populations of Merodon aureus and M. cinereus species complexes (Diptera, Syrphidae): integrative taxonomy and implications for conservation priorities on the Balkan Peninsula. *Conserv. Genet.* **9**: 1125–1137.

MILANKOV V., LUDOŠKI J., STÅHLS G., STAMENKOVIĆ J. & VUJIĆ A. 2009: High molecular and phenotypic diversity in the Merodon avidus complex (Diptera, Syrphidae): cryptic speciation in a diverse insect taxon. *Zool. J. Linn. Soc. Lond.* **155**: 819–833.

MILANKOV V., FRANCUSKI L., LUDOŠKI J., STÅHLS G. & VUJIĆ A. 2010: Estimating genetic and phenotypic diversity in a northern hoverfly reveals lack of heterozygosity correlated with significant fluctuating asymmetry of wing traits. *J. Insect Conserv.* **14**: 77–88.

MØLLER A.P. 1993: Developmental stability, sexual selection and speciation. *J. Evol. Biol.* **6**: 493–509.

MONTEIRO F., LAZOSKI C., NOIREAU F. & SOLE-CAVA A. 2002: Allozyme relationships among ten species of Rhodniini, showing paraphyly of Rhodnius including Psammolestes. *Med. Vet. Entomol.* **16**: 83–90.

MUNSTERMANN L.E. 1979: *Isozymes of Aedes aegypti: Phenotypes, Linkage, and Use of Genetic Analysis of Sympatric Population in East Africa.* PhD. Thesis, University of Notre Dame, Indiana.

NEI M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

OLIVIERI G.L., SOUSA V., CHIKNI L. & RADESPIEL U. 2008: From genetic diversity and structure to conservation: genetic signature of recent population declines in three mouse lemur species (Microcebus spp.). *Biol. Conserv*. **141**: 1257–1271.

PALMER A.R. 1994: Fluctuating asymmetry analyses: A primer. In Markow T.A. (ed.): *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer, Dordrecht, pp. 335–364.

PALMER A.R. & STROBECK C. 1986: Fluctuating asymmetry: measurements, analysis, patterns. *Annu. Rev. Ecol. Syst.* **17**: 391–421.

PALMER A.R. & STROBECK C. 1992: Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power statistical tests. *Acta Zool. Fenn.* **191**: 57–72.

PASTEUR N., PASTEUR G., BONHOMME F., CATALAN J. & BRITTON-DAVIDIAN J. 1988: *Practical Isozyme Genetics*. Ellis Horwood, Chichester, 215 pp.

REED D.H. & FRANKHAM R. 2003: Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**: 230–237.

ROHLF F.J. 2004: *tpsDig – Thin Plate Spline Digitizer, version 1.40.* Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

ROHLF F.J. 2005: *tpsRegr – Thin Plate Spline Shape Regression, version 1.31.* Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

ROHLF F.J. 2006: *tpsRelw – Thin Plate Spline Relative Warp, version 1.44*. Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

ROHLF F.J. & SLICE D. 1990: Extensions of the procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**: 40–59.

ROHLF F.J., LOY A. & CORTI M. 1996: Morphometric analysis of old world talpidae (Mammalia, Insectivora) using partial-warp scores. *Syst. Biol*. **45**: 344–362.

SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H. & FLOOK P. 1994: Evolution, weighing, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**: 651–701.

SMITH D.R., CRESPI B.J. & BOOKSTEIN F.L. 1997: Fluctuating asymmetry in the honeybee, Apis mellifera: effect of ploidy and hybridization. *J. Evol. Biol.* **10**: 551–574.

SOTO I.M., CARREIRA V.P., SOTO E.M. & HASSON E. 2008: Wing morphology and fluctuating asymmetry depend on the host plant in cactophilic Drosophila. *J. Evol. Biol.* **21**: 598–609.

STÅHLS G., RIBEIRO E. & HANSKI I. 1989: Fungivorous Pegomya flies: spatial and temporal variation in a guild of competitors. *Ann. Zool. Fenn.* **26**: 103–112.

STÅHLS G., VUJIĆ A., PÉREZ-BAÑÓN C., RADENKOVIĆ S., ROJO S. & PETANIDOU T. 2009: COI barcodes for identification of Merodon hoverflies (Diptera, Syrphidae) of Lesvos Island, Greece. *Mol. Ecol. Res.* **9**: 1431–1438.

STIGE L.C., DAVID B. & ALIBERT P. 2006: On hidden heterogeneity in directional asymmetry – can systematic bias be avoided? *J. Evol. Biol.* **19**: 492–499.

SWOFFORD D.L. & SELANDER R.B. 1989: *BIOSYS-2: A Computer Program for the Analysis of Allelic Variation in Genetics. Release 1.7. Users Manual.* Illinois Natural History Survey, Champaign.

TROTTA V., CALBOLI F.C.F., GAROIA F., GRIFONI D. & CAVICCHI S. 2005: Fluctuating asymmetry as a measure of ecological stress in Drosophila melanogaster (Diptera: Drosophilidae). *Eur. J. Entomol.* **102**: 195–200.

VAINIO J. & VÄINÖLÄ R. 2003: Refugial races and postglacial colonization history of the freshwater amphipod Gammarus lacustris in Northern Europe. *Biol. J. Linn. Soc.* **79**: 523–542.

VAN DONGEN S. 2006: Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. *J. Evol. Biol.* **19**: 1727–1743.

VAN DONGEN S., LENS L., PAPE E., VOLCKAERT F.A.M. & RAEYMAEKERS J.A.M. 2009: Evolutionary history shapes the association between developmental instability and population-level genetic variation in three-spined sticklebacks. *J. Evol. Biol.* **22**: 1695–1707.

VAN VALEN L. 1962: A study of fluctuating asymmetry. *Evolution* **16**: 125–142.

VØLLESTAD L.A., HINDAR K. & MØLLER A.P. 1999: A meta-analysis of fluctuating asymmetry in relation to heterozygosity. *Heredity* **83**: 206–218.

WILLMORE K.E., YOUNG N.M. & RICHTSMEIER J.T. 2007: Phenotypic variability: its components, measurement and underlying developmental processes. *Evol. Biol.* **34**: 99–120.

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