Detecting Intraspecific Character Displacement by Morphological Markers in Riverine-Dwelling Invertebrate Larvae: The Case Study of Head Shape Variability in *Leuctra fusca* (Plecoptera: Leuctridae)

Raffaella Bravi, Lorenzo Traversetti* and Massimiliano Scalici

Department of Sciences, University of Roma Tre, Viale G. Marconi 446, 00146, Rome, Italy

Abstract: Since morphological markers are recognized as useful tools to evaluate events of anthropic disturbances, we performed a preliminary study on head shape variability in the riverine-dwelling *Leuctra fusca* larvae as early alarm systems in running waters. Particularly, heads of 32 larvae were collected from two localities of River Aniene (central Italy) and photographed for digitizing landmarks and semilandmarks. The Cartesian x-y coordinates of all points were firstly converted to shape coordinates by Procrustes superimposition, and then analyzed for exploring the full potential of the application of geometric morphometric techniques. Where the principal component analysis revealed a clear pattern of variation between the 2 sampling sites, the Procrustes ANOVA highlighted this variation as highly associated with fluctuating asymmetry, the latter being traditionally connected with developmental accidents due to environmental conditions. No directional asymmetry was observed. Finally we didn't find any pattern of allometric variation in the studied structure. Our study indicates that further studies ought to be employed to use geometric morphometrics as a valid tool for detecting and describing morphological variation as biomarkers in invertebrate organism such as stoneflies.

Keywords: Asymmetry, biomarker, central Italy, freshwater, geometric morphometrics.

INTRODUCTION

Both directional (DA) or fluctuating (FA) asymmetries are widely recognized as morphological markers of anthropic disturbances [e.g., 1] in riverine macroinvertebrates [see 2, 3] for assessing river water quality [4]. DA occurs whenever one character developed more in one side of the plane or planes of symmetry than in the other, while FA is defined as those random differences which occur between the left and right sides in a normally symmetrical organism [5]. Thus, FA has been used as an marker of the level of developmental stability and in defining the influence of both environmental and genetic stress on development [4, 5].

Here we are aimed to explore the potential of morphological biomarkers in assessing the head shape modification in larval specimens of the stonefly *Leuctra fusca* (Linnaeus, 1758) known as a up- and middle-stream dwelling organism, inhabiting areas with no and/or low anthropic disturbance. The *L. fusca* larvae are considered as ideal organisms for environmental monitoring activities as they (1) colonize different habitat being one of the most tolerant organism in central Italy amongst Plecoptera (the latter being considered the most sensitive order of invertebrates on the whole), and (2) spend most of their developmental time in the sediment surface, where they remain exposed to different stressors, as other species of the same genera [6].

MATERIALS AND METHODS

We analyzed and photographed 32 *L. fusca* specimens quantitatively collected in April 2011 in 2 different sites (Ani1 and Ani3) of the River Aniene (central Italy). Ani1 is the upper site located on the river, almost at 5 km from the sources. This site is characterized by a great naturality and a low visible amount of human impacts, mainly related with pasture and agriculture [7, 8]. On the contrary, Ani3 is located near Anticoli Corrado and the presence of wastewaters and other human infrastructures show an higher anthropization in respect to Ani1 [7, 8]. We then digitized 6 landmarks (LMs) and 6 semilandmarks (SLs, points with a reduced degree of freedom depending upon landmarks) on the pictures of the larval head (calibrated against graph paper to standardize measurements) by TpsDig 2.10 [9] (Figure **1a**). All following analyses were run by the MorphoJ software version 1.05a [10]. The shape information was extracted from the coordinate data by using a full Procrustes fit [11], that removes information on location orientation and rotation, and standardizes each specimen to unit centroid size (CS, that is, the square root of the summed squared Euclidean distances from each landmark to the specimen centroid that provides an estimate of the size of the studied structure). We firstly analyzed shape variation in the entire data set by using an explorative Principal Component Analysis (PCA), based on the covariance matrix of symmetric

*Address correspondence to this author at the Department of Sciences, University of Roma Tre, viale G. Marconi 446, 00146, Rome, Italy;
Tel: +390657336357; Fax: +390657336321;
E-mail: lorenzo.traversetti@uniroma3.it

ISSN: 1814-8085 / E-ISSN: 1927-5129/14

and asymmetric components of the shape variation. We then correlated the log-transformed CS with PCs for providing an eventual significant size-dependent variability of the head shape changes. Additionally, we analyzed the amount of symmetric variation and asymmetry using Procrustes ANOVA as assessed for studies on object symmetry [12]. To avoid the assumption of having an equal and independent variation at all points, we performed a MANOVA test for symmetric component and asymmetry. Finally, differences between the two sample sites were assessed by Canonical Variate Analysis (CVA), used to find the shape characters that best distinguish among multiple groups of specimens. The results of the analysis are reported as both Mahalanobis and Procrustes distances, which are multivariate measures of distance relative to the within sample variation.

RESULTS AND DISCUSSION

The present study aimed to identify intraspecific morphological variation in the head of *L. fusca* between two populations inhabiting two distant sites of the same Mediterranean river, and to detect significant variations in the FA patterns in these two sites.

The PCA scatter plot for the symmetric component showed a great variability of the whole sample and a significant differences between the two analyzed populations (Figure **1b**). The PCA scatter plot for asymmetric component instead did not show differences between the two populations (Figure **1c**). Then, we only showed the symmetric component of the PCA for describing the individual shape variations among the entire sample (Figure **1d**).

As for the allometry, the multivariate regression of head shape vs. log-transformed CS accounted 46.67% of the shape variation, indicating the absence of allometry in the analyzed sample, finding confirmed by the permutation test (10,000 permutation runs, P = 0.14).

Procrustes ANOVA showed significant differences between the two sampled sites Ani1 and Ani3, for both size and shape (Table **1**), showing a significant pattern of FA of the entire sample. MANOVA test confirmed these results for both symmetric variation (Pillay = 0.64, P = 0.0075) and asymmetry (Pillay = 0.64, P = 0.0057). Finally a great morphological variability emerged from CVA (Figure **2**) and the differences among the two populations of Ani1 and Ani3 were significant (Mahalanobis distance = 2.65, P< 0.0001; Procrustes distance = 0.07, P = 0.0008).

FA emerged to be well evident in the head of *L. fusca* in both size and shape components of the variation in the whole sample, and did not resulted affected by allometry in the two sites. Regarding sites, Ani1 had the greater amount of morphological variability in a little size range; on the reverse, Ani3 had wide dimensional variability in a little morphological range. These findings were greater than expected by chance indicating a certain amount of environmental instability, mostly concentrated in Ani1. At the first glimpse, our results could be apparently discordant with what emerged from literature [2, 7, 13, 14] especially regarding anthropogenic impact, since FA is commonly considered as a symptom of environmental noise [5]. The great morphological variability observed in Ani1 may be affected by the reduced selective pressure due to predators, as this site is nearest to the river source than Ani3 [15]. In particular, fish predation can reduce the survival of more asymmetric larvae (that are, specimens with a higher level of disturbance on functional characters than more symmetric individuals).

Table 1: Procrustes ANOVA for both shape and size for head, a structure characterized by object symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless)

		SS	MS	df	F	P(param)
Centroid size	population	0.153	0.153	1	16.58	0.0003
	individual	0.268	0.00925	29		
Shape	population	3.266	327	10	609	<0.0001
	individual	15.553	54	290	223	<0.0001
	side	417	42	10	174	723
	ind * side	7.201	24	300	-	-

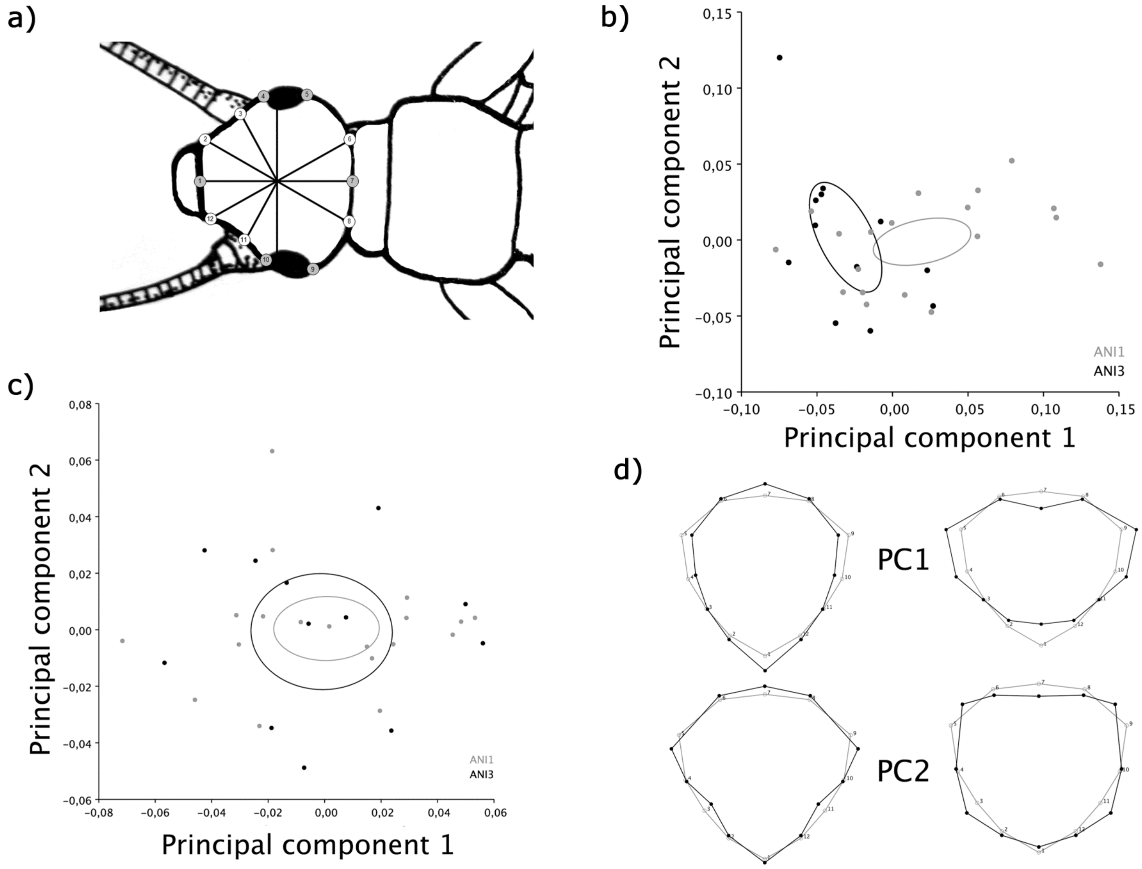

Figure 1: a) landmarks (gray) and semilandmarks (white) digitized on the analyzed specimens; **b**) principal component analysis for the symmetric components of the two first principal components; **c**) principal component analysis for the asymmetry component of the two first principal components; **d**) shape changes associated with firsts two PC axes (starting shape is shown with the grey line and target shape with the black one). For each of the two PCs the black line shows the shape deformation from right to left, corresponding to the observed extremes of the PCA axes, from negative to positive directions.

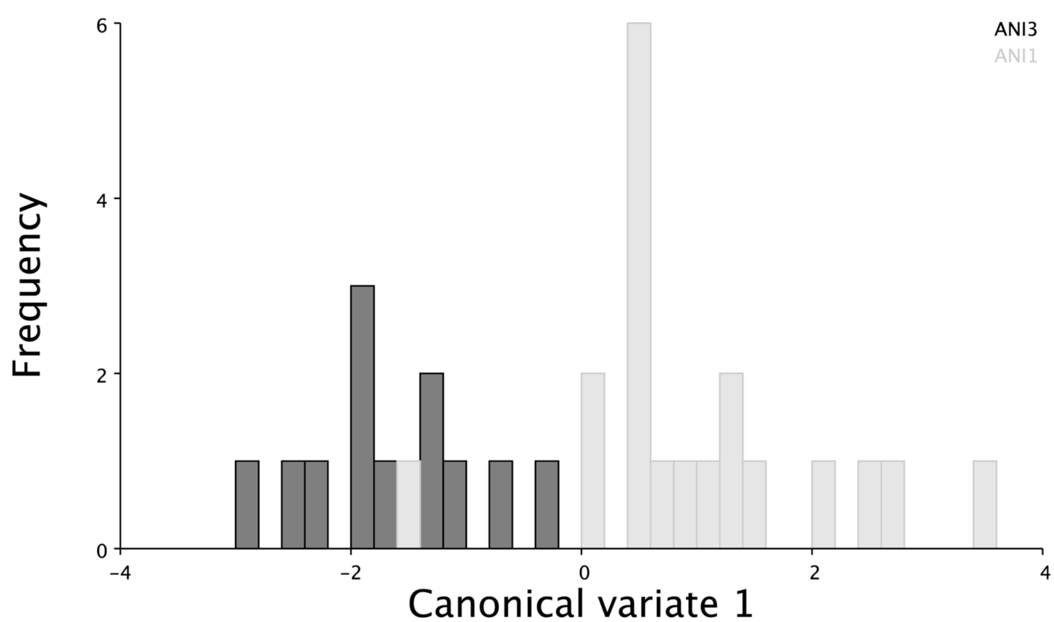

Figure 2: Canonical variate analysis, showing the differences in the two sites, Ani1 and Ani 3 computed for whole investigated sample.

This work represents an example of how geometric morphometrics may be used to identify such anatomical differences among populations. More information on the expression of different kinds of factors is clearly needed to use biomarkers or FA efficiently in detecting early stressors' impact. This type of study may provide a good baseline to link observed character displacement with functional and biomechanical changes with the tool of geometric morphometric. Moreover, our results suggest that FA may be reliable in detecting biological changes even in two apparently similar sites, as Ani1 and Ani3 are. More data are required in order to evaluate the actual response of body-form-related descriptors to external stressors.

REFERENCES

[1] Møller AP, Swaddle JP. Asymmetry, Developmental Stability, and Evolution. Oxford: Oxford University Press 1997.

[2] Bonada N, Williams DD. Exploration of the utility of fluctuating asymmetry as an indicator of river condition using larvae of the caddisfly *Hydropsyche morosa* (Trichoptera: Hydropsychidae). Hydrobiologia 2002; 481: 147-156. http://dx.doi.org/10.1023/A:1021297503935

[3] Al-Shami SA, Che Salmah MR, Hassan AA, Siti Azizah MN. Fluctuating asymmetry of *Chironomus* spp. (Diptera: Chironomidae) larvae in association with water quality and metal pollution in permatang Rawa river in the Juru river basin, Penang, Malaysia. Water Air Soil Pollut 2011; 216: 203-216. http://dx.doi.org/10.1007/s11270-010-0528-4

[4] Clarke GM. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. Environ Pollut 1993; 82: 207-211. http://dx.doi.org/10.1016/0269-7491(93)90119-9

[5] Van Valen L. A study of fluctuating asymmetry. Evolution 1962; 16: 125-142. http://dx.doi.org/10.2307/2406192

[6] Campaioli S, Ghetti PF, Minelli A, Ruffo S. Manual for the identification of Italian freshwater macroinvertebrates. Trento, Italy, Trento Autonomous Province 1999.

[7] Manfrin A, Larsen S, Traversetti L, Pace G, Scalici M. Longitudinal variation of macroinvertebrate communities in a Mediterranean river subjected to multiple anthropogenic stressors. Int Rev Hydrobiol 2013; 98: 155-164. http://dx.doi.org/10.1002/iroh.201201605

[8] Traversetti L, Scalici M, Ginepri V, Manfrin A, Ceschin S. Concordance between macrophytes and macroinvertebrates in a Mediterranean river of central Apennine region. J Environ Biol 2014; 35: 497-503.

[9] Rohlf FJ. TpsDig Ver. 2.10. Digitalized Landmarks and Outlines. State University of New York, Department of Ecology and Evolution, Stony Book 2006.

[10] Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 2011; 11: 353-357. http://dx.doi.org/10.1111/j.1755-0998.2010.02924.x

[11] Dryden IL, Mardia KV. Statistical Shape Analysis. Chichester, UK, Wiley 1998.

[12] Klingenberg CP, Monteiro LR. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst Biol 2005; 54: 678-688. http://dx.doi.org/10.1080/10635150590947258

[13] Chang X, Zhai B, Wang M, Wang B. Relationship between exposure to an insecticide and fluctuating asymmetry in a damselfly (Odonata, Coenagriidae). Hydrobiologia 2007; 586: 213-220. http://dx.doi.org/10.1007/s10750-007-0620-y

[14] Chang X, Zhai B, Liu X, Wang M. Effects of temperature stress and pesticide exposure on fluctuating asymmetry and mortality of *Copera annulata* (Selys) (Odonata: Zygoptera) larvae. Ecotoxicol Environ Saf 2007; 67: 120-127. http://dx.doi.org/10.1016/j.ecoenv.2006.04.004

[15] Dodds W. Freshwater Ecology. Philadelphia, USA, Academic Press 2010.

Received on 31-03-2014 Accepted on 23-06-2014 Published on 18-07-2014

http://dx.doi.org/10.6000/1927-5129.2014.10.41