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Non-invasive methods for morphometric analyses of lepidopteran wings

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Summary: Butterfly wings are commonly used in morphometric studies. For this reason they are detached from the body in order to get a clearer image for the analysis. In some cases this is not desirable as it would destroy a butterfly that may have greater value displayed. Our results show that displayed butterflies can also be used in morphometric analyses.

Key words: butterfly, wing, morphometrics, displayed, detached, *Melanargia galathea.*

Introduction

Geometric and traditional morphometric measurements (Zelditch *et al.* 2004) have been used successfully in many studies of wing shape and wing pattern variation among butterflies (Descimon & Renon 1975, Luebke *et al.* 1988, Dudley 1990, Monteiro *et al.* 1997, Winding *et al.* 2001, Breuker *et al.* 2010, Dincă *et al.* 2011, Gibbs *et al.* 2011, Jorge *et al.* 2011, Cespedes *et al.* 2014, Habel *et al.* 2016, Martin *et al.* 2016). Modern technology could even allow automatisation of such forms of analysis (Houle *et al.* 2003). These measurements are often done on detached wings, as they are easier to manipulate and the risk of error is reduced. Live specimens can be photographed in the field and then released with the method developed by Nève & Descimon (2005), so the problem arises when dealing with displayed butterflies, belonging to a collection or an endangered species, which would be damaged by wing detachment.

For this reason we attempted to determine if there are statistically significant differences between morphometric measurements of detached and displayed wings. *Melanargia galathea* (Linnaeus, 1758) butterflies were used as they are a common, non-endangered species and their wings have clear patterns that make landmarks easy to identify.

Materials and methods

Melanargia galathea butterflies were caught in June 2016 near the city of Răscruci (Cluj county, Romania), they were later displayed on a wooden support. Hairs close to the base of the wings were removed for a clearer picture. An insect pin was used to hold the butterfly in a still position while displayed. 10 male *M. galathea* butterflies were used in the analysis, in order to avoid any issues caused by sexual dimorphism. Three sets of measurements were made.

For the first analysis we tested if there was variation due to human error while placing the displayed butterflies on the stand to be photographed. The ten butterflies were photographed in two separate sessions, forming two groups of pictures.

For the second analysis we tested whether there was a difference at an individual level between displayed and detached wings. To do this a single butterfly was photographed ten times with the wings attached and then another ten times with the wings detached, these were the two groups of pictures then compared.

The third analysis was similar to the second but with all ten butterflies. They were first photographed with the wings attached and then with the wings detached, the two sets of pictures making the two groups used for comparison.

The displayed butterflies were photographed on graph paper, with great care taken so that the insect pin passing through the thorax was at a perpendicular angle to the wings. The Canon EOS 500D camera was placed on a tripod for a fixed position. The detached wings were photographed a second time, placed over graph paper and through a stereomicroscope using an Optika M HDMI 5 MP camera.

The digital photographs were converted to the TPS format using the software TPSUtil, which allows them to be processed in a system of coordinates. Landmarks (Bookstein 1986) were placed with the program TPSDig2 (http://life.bio.sunysb.edu/morph/soft-utility.html), the placement was at the

base of the wing at the split of the main vein and on the intersection of wing veins with the wing edge, the right forewing was the one analyzed (Fig. 1). Differences among individuals was tested using Principal Component Analysis, as this method reduces the dimensionality of multivariate data and allows easy graphical representation using the axes that cover the majority of the variation. The statistical differences were calculated using a Permutation test as our values might not follow a normal distribution and this test can reach a conclusion without assuming any distribution (Zelditch *et al.* 2004). Principal Component Analysis (PCA) and the Permutation Test were calculated using the program MorphoJ (http://www.flywings.org.uk/morphoj_page.htm).

Results

The first analysis, employing ten displayed butterflies photographed twice, showed no statistically significant value for the permutation test: p=0.9947 after 10 000 permutations. Principal component analysis (PCA) also shows a fairly homogenous distribution (Fig. 2).

The second analysis, the wings of the same individual photographed ten times displayed and then detached, had a statistically significant result: p<0.0001 for the permutation test after 10 000 permutation. PCA (Fig. 3) shows clustered distributions for the two groups.

The third analysis, the wings of ten individuals photographed displayed and then detached, did not show a statistically significant difference: p=0.7768 after 10 000 permutations. Once again PCA (Fig. 4) shows an overlapped distribution.

Discussion

Our results show that displayed butterflies can be used in morphometric comparisons but only under certain conditions. Great care has to be taken when preparing the material for photography. In our case the greatest variation could have been given by the angle of the wings with the camera lens, by making sure that the wings were parallel to the base we obtained homogenous photographs that were not statistically different from each other, as shown by our first analysis.

Our second and third comparisons showed that variation when comparing displayed wings to detached wings is significant only when the sample size is small.

In conclusion we can affirm that, while detached wings are preferred for morphometric studies, displayed wings can be used if the situation demands it as long as large sample sizes are used. These results

Fig. 1. Landmark placements on the right wing of a displayed male *Melanargia galathea* butterfly.

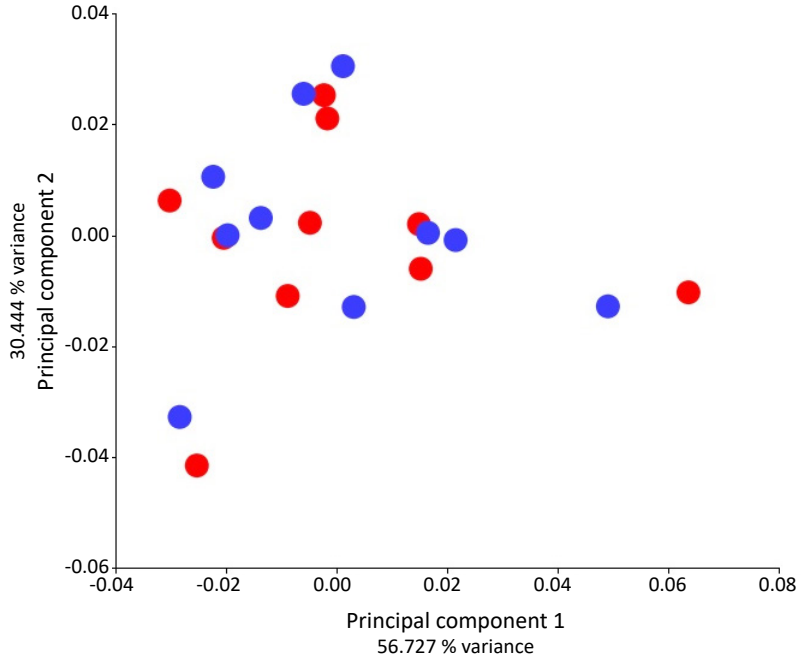

Fig. 2. First and second PCA axes for the first analysis covering 87.171 % of the variance, ten displayed butterflies photographed twice, with blue dots representing results from the first set of photographs and red from the second set.

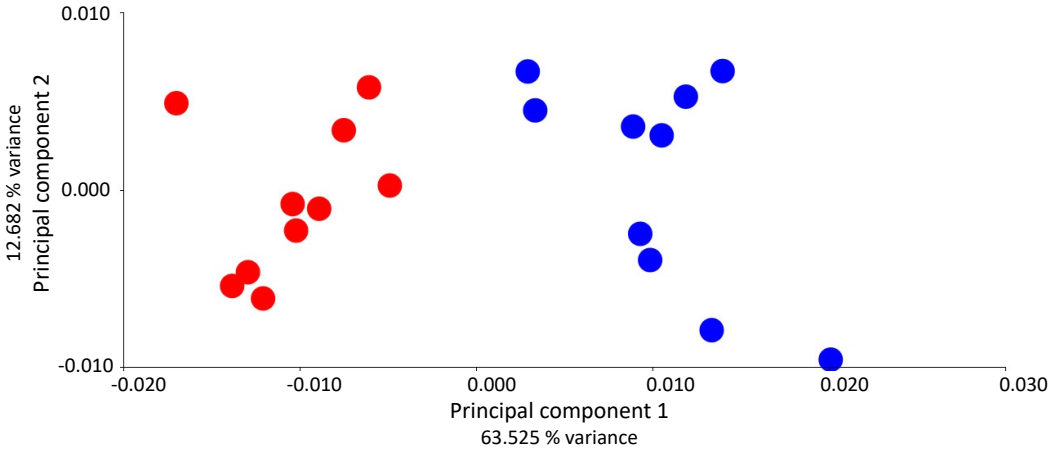

Fig. 3. First and second PCA axes for the second analysis covering 76.206 % of the variance, the wings of the same individual photographed ten times displayed (blue) and then detached (red).

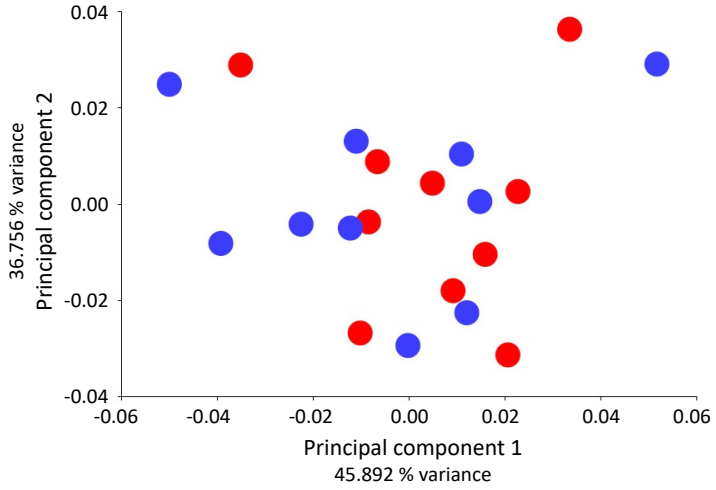

Fig. 4. First and second PCA axes for the third analysis covering 82.648 % of the variance, the wings of ten individuals photographed displayed (blue) and then detached (red).

show that non-invasive methods can be used in morphometric studies on lepidopteran wings, sparing the live of the individuals used in the analysis or protecting valuable pieces from collections.

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