Shape Analysis of Blow Fly (Diptera: Calliphoridae) Wing Venation

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Abstract

A new method of identification of blow flies is geometric morphometric of the blow fly wing venation. In this study, 83 specimens form 5 different species were run through the MorphoJ software. The results showed that geometric morphometrics can be used to identify easily to genus, and in some cases, it can be used to identify to species.

Introduction

Forensic entomology is the use of insects in the legal system. One of the most common ways is in post mortem interval (PMI) estimates. The PMI estimate method that is most well-known is the use of Accumulated Degree Days or Hours (ADD or ADH) (Reibe 2010). The ADH represents the number of hours needed for the development of the blow fly larvae. The ADD or ADH concept relies on the assumption that the developmental rate is proportional to the temperature within a certain species-specific temperature (Reibe 2010). It is important to know the exact species of the fly to estimate the correct PMI. The current types of identification are morphologically using taxonomy or DNA testing. DNA testing can be expensive and not everyone has the testing capabilities. Morphological identification is not always accurate and does not have a confidence interval. Geometric morphometrics of the blow fly wing venation is a new option for identification. It is a quantitative species identification and would provide a confidence interval. This is important because part of the *Daubert* standards is that there is a

known or potential error rate. Currently there is not, but geometric morphometrics can provide one.

Geometric morphometrics is the statistical analysis of shape variation (Adams 2012). It involves finding locations called landmarks. In this case the landmarks on the wing venation of blow flies. A Procrustes analysis is then applied to the landmarks to remove any non-shape variation. The Procrustes analysis scales to the same size, shifts to the same position, and rotates to the same orientation (Adams 2012). Geometric morphometrics should be able to show if there is a difference between the landmarks of blow fly wing venation. I hypothesize that there will be a difference in the shape of the wing venation between species.

Materials and Methods

A total of 115 specimens were pulled from the 2014, 2015, 2016 ICFOD data. There were 22 Phormia regina specimens, 23 Lucilia illustris specimens, 20 Cochliomyia macellaria specimens, 25 Lucilia coeruleviridis specimens, and 25 Lucilia sericata specimens. The extra specimens were to try to guarantee 20 good specimens for each species. The right wing was removed from each specimen using fine-tipped forceps. I griped the wing at the base and gently pulled until the wing was removed from the body of the fly. I then placed a drop of Elmer's glue on a glue board. I spread the glue out so that the glue was in a thin layer on the board. I then used a pair of featherweight forceps to place the wing ventral side up on the glue board. I then pressed to wing lightly to make sure that it would stay and was flat on the board. All of the wings where photographed using a Leica M165C microscope. All of the specimens were photographed with a 5millimeter scale. After photographing, there were 15 good L. coeruleiviridis specimens, 16

good L. illustris specimens, 18 good C. macellaria specimens, 17 good L. sericata specimens, and 17 good P. regina specimens.

I used tpsUTIL32 to change the photographs from jpeg to a TPS file. I then used the TPS file to input my landmarks using the tpsDIG232 software. First, I went to image tools and selected measure to set the program scale to the same scale used in the image. The scale was set to 274 pixels per millimeter. I then used the crosshairs to mark my ten landmarks (Fig. 1). This was repeated for every image. Once all of the landmarks were input, I uploaded the data into MorphoJ (Klingenberg 2011). The first thing done in MorphoJ was to perform a Procrustes Superimposition. I did this by going to the preliminaries menu and selecting "new Procrustes fit." This shows a graph with all of the landmarks plotted in black and with blue dots that are the average for each landmark. To generate a covariance matrix, I went back to the project tree and selected the data wanted. Under the preliminaries menu, generate covariance matrix was selected. Back in the project tree, a CovMatrix for that dataset is now visible. I selected the CovMatrix and then clicked on the Variation tab to get to Principal Component Analysis. This produces a graph that shows the average of the landmarks and the direction and size of the differences. I ran this for all individual species and a combined species dataset. Under the comparison menu, I performed a Canonical Variance Analysis, selecting my data, data type, and the way I wanted to compare my data. I then ran the test at 10,000 permutations.

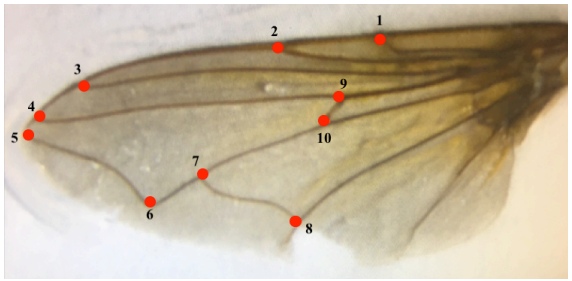

Fig. 1 Right wing with 10 plotted landmarks.

Results

A principle component analysis (PCA) was created to show to representation of shape similarity relations (MacLeod, 2018). The PCA (Fig. 2) shows the shape distribution for all of the species used in the study. The specimens are plotted along the first two principal component axes (principal component 1 and principal component 2). The species is starting to break apart by subfamily with Chrysomyinae on the left of principal component 1 and Luciliinae on the right of principal component 1.

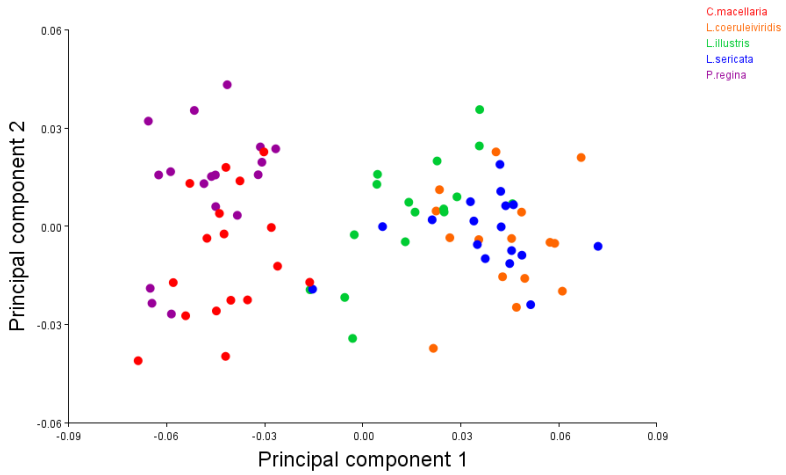

Fig. 2 The principal component analysis for all five species.

A Canonical Variate Analysis (CVA) was run after the PCA, to maximize variation between the groups and minimize intraspecific variation (Sontigun 2017). I first ran this test with just the genera of the specimens (Fig. 3). Like the PCA the CVA was plotted along the first two canonical variate axes (canonical variate 1 and canonical variate 2). The genera each separated out to their own area. The genus Cochliomyia was on the right side of canonical variate 1 and the top of canonical variate 2. The genus Lucilia was on the left of canonical variate 1 and at the top of canonical variate 2. The genus Phormia was in the middle of canonical variate 1 and at the bottom of canonical variate 2.

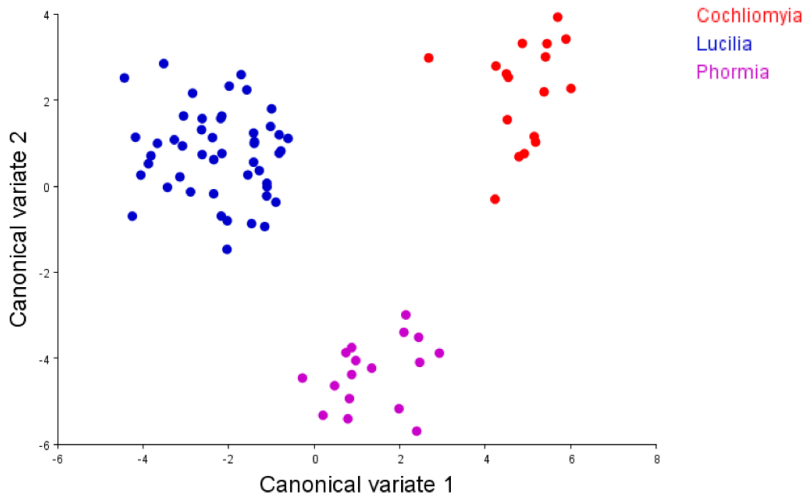

Fig. 3 The canonical variate analysis separated by genera.

Another CVA was run with the same axes. This CVA includes all 5 species of the study (Fig. 4). Cochliomyia macellaria is on the left side of canonical variate 1 and on the bottom of canonical variate 2. Phormia regina is on the left side of canonical variate 1 and at the top of canonical variate 2. Lucilia illustris is near the middle of canonical variate 1 and canonical variate 2. Lucilia sericata and Lucilia coeruleiviridis are on the left side of canonical variate 1 and the middle of canonical variate 2.

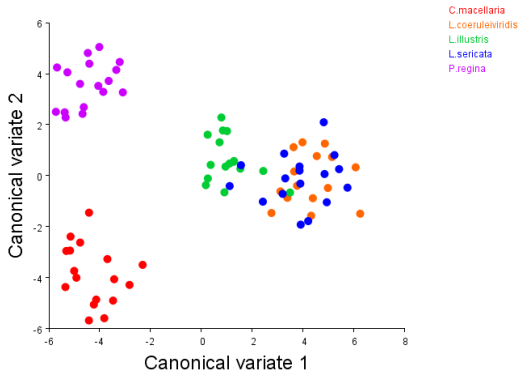

Fig. 4 The canonical variate analysis including all 5 species.

Discussion

The CVA results show that wing shape can be separated out by genus for all of the genera in this study. The results also support the identification to species for three of the five species in the study. The supports my hypothesis that there is a difference in the shape of the wing venation between species. There is no surprise in the overlap of L. coerulevirids and L. sericata. The two are difficult to distinguish morphologically, and molecular studies show that there is low interspecific variation between the closely related species (Sontigun 2017). The DNA identification can be costly. Geometric morphometric analysis is a good substitution to help with the difficulty of identification for some species (Sontigun 2017). The use of more unique landmarks on the wing may help separate out the closely related species. More work needs to be done to make this a more common form of identification.

Acknowledgment

I would like to thank Dr. Trevor Stamper for the use of his lab and for the idea for this project. I would also like to thank Greg Nigoghosian for his assistance with the methods and results section of this study. Other acknowledgments go to Kelie Yoho, Brianna Pennell, and Caydee Terrell.

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