The relationship between wing color pattern, wing area, and wing weight in *Celithemis* dragonflies based on the Targeted Odonata Wing Digitization (TOWD) image database

Ahmed Elmonier, Essex County College, Dr. Dirk Gassmann, Rutgers University, Newark, Dr. William R. Kuhn,
University of Tennessee, Prof. Dr. Jessica L. Ware, Rutgers University, Newark.
Department of Biology – Rutgers University, Newark

Abstract

The evolution of dragonfly wings is subjected to both natural and sexual selection pressures. The Targeted Odonata Wing Digitization (TOWD) aims at scanning the wings of all North American dragonfly species. Using the genus *Celithemis* Hagen, 1861, as an example, we demonstrate the potential of this unique image database for quantifying and analyzing wing parameters in the Odonata. Based on area measurements of wings and wing spots taken from wing scans mainly using standard imaging software, and on wing weight measurements of selected *Celithemis* species, we established basic relationships of these parameters for the genus and discuss them with regard to the ecology and behavior of the species. Future research should focus on a wider range of species with similarly colored wings and should consider further characters (e.g. wing shape) to determine the evolutionary factors acting on the dragonfly wing.

Introduction

Dragonflies (Odonata) are ancient semi-aquatic insects. Due to their excellent flying abilities, the odonate wing morphology is of great interest to scientists. The Targeted Odonata Wing Digitization (TOWD) Project is an effort to digitize the wings of the 466 species of North American dragonflies and damselflies (Odonata), building a comprehensive library of high-resolution imagery of these species. These data will be used to (1) update our species recognition system for Odonata, called Odomatic, for distinguishing North American species, and (2) to create a rich database of morphological characters for studies of their evolutionary biology. During measurements of wing weight across different groups of libellulid dragonflies, it became apparent that wing coloration might contribute to wing weight. Using the genus *Celithemis*, which in all species exhibits some kind of wing coloration, we here present a first approach to testing the **hypothesis** that the increase in the extent of dragonfly wing coloration leads to an increase in wing weight.

Figure 1: Male *Celithemis elisa*
Photo:
John C. Abbott/OdonataCentral

Figure 2:
Male *Celithemis eponina*
Photo: William R. Kuhn

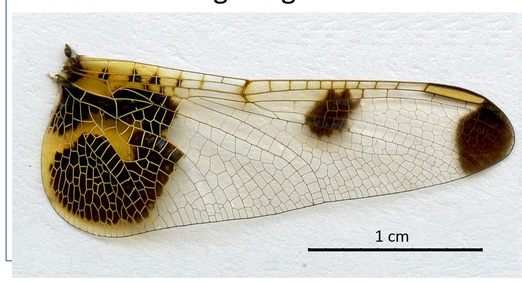

Figure 3: Right hindwing *of C. elisa* ♂

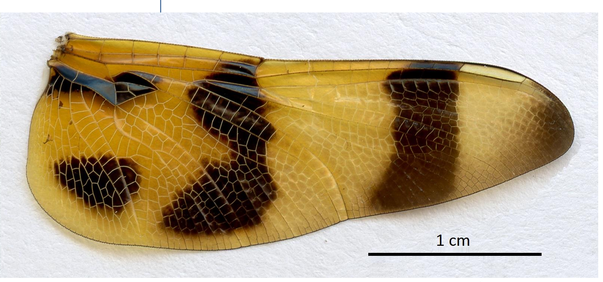

Figure 4: Right hindwing *of C. eponina* ♂

Methods and Materials

Right fore- and hindwings, respectively, of male specimens of *Celithemis elisa* and *C. eponina* were dissected from the body, scanned in high resolution (1200 dpi) by means of a standard flatbed scanner and uploaded to the Bisque image database (http://bisque.cyverse.org). From there, wing area measurements were calculated automatically using a segmentation algorithm by Kuhn (2016). For quantifying the extent of coloration, the single dark-brown wing spots on the hindwings of both species were marked manually and selected using the “Path” Tool in the imaging software GIMP 2.10.6. The pixel counts (rendered by the program) of all wing spots were summed up to yield the total wing spot pixel count. The latter was calibrated based on the scanned scale and transformed into area units (mm^2) to get the total wing spot area for each wing. For statistical analysis, the latter was rescaled by dividing it by the respective wing area. Bivariate plots and calculation of the correlation parameters were performed with the statistics program R.

Hindwings were weighed with a precision of 0.0001 g using an analytical balance (A-200D, Denver Instrument Company). With all parameters included, we got a sample size of n = 18 for *C. elisa* and one of n = 9 for *C. eponina. W*e calculated the Pearson coefficient *r* of correlation (normality tested after Shapiro-Wilk).

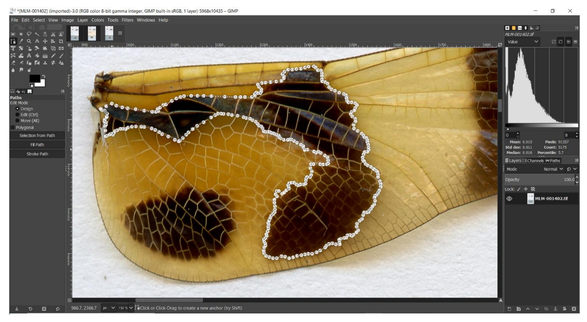

Figure 5: *Celithemis* hindwing spot marked with GIMP

Results

For rescaled total wing spot area vs. wing weight, we obtained Pearson correlation coefficients of *r* = -0.4621586 *(C. elisa,* p =0.05349; Fig. 6)), and *r* = -0.5989413 (*C. eponina,* p = 0.08833; Fig. 7). For wing area vs. wing weight, we obtained correlation coefficients of *r* = -0.1534597 *(C. elisa,* p =0.6167; not shown here)), and *r* = 0.4267448 (*C. eponina,* p = 0.252; Fig. 8).

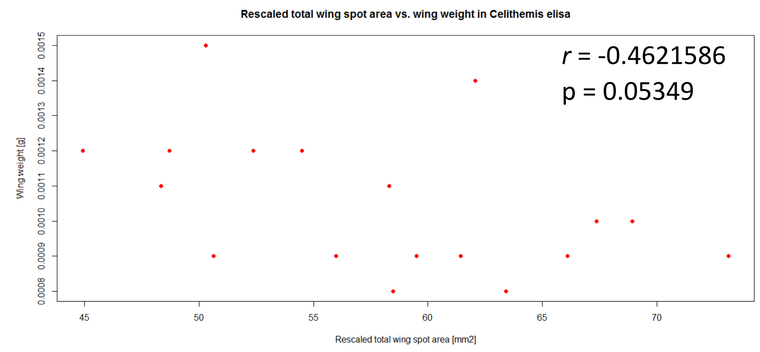

Figure 6: Bivariate plot of rescaled total wing spot area vs. wing weight in *C. elisa*.

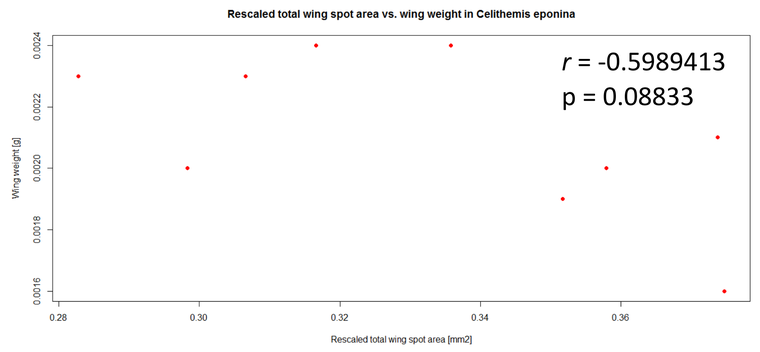

Figure 7: Bivariate plot of rescaled total wing spot area vs. wing weight in *C. eponina*.

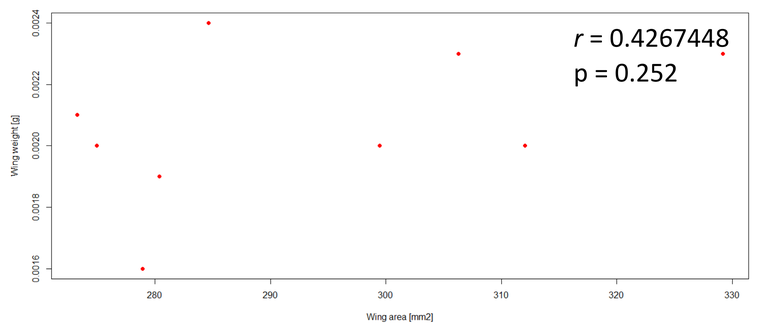

Figure 8: Bivariate plot of wing area vs. wing weight (only shown here for *C. eponina*)

Discussion

The correlation coefficients show a moderate negative correlation of rescaled total wing spot area and wing weight in both *C. elisa* and *C. eponina*. With a p-value of about 0.05, only the *C. elisa* dataset yields a significant correlation. For the relationship wing area vs. wing weight, we got a weak negative correlation for *C. elisa*, while for *C. eponina* a positive moderate correlation coefficient was obtained; in both cases, correlation was not sigificant. Since due to the presumed deposition of epidermal pigment in the dark areas of *Celithemis* wings we expected wing weight to increase with an increasing degree of coloration, the unexpected negative correlation between rescaled total wing spot area and wing weight could point to deficiencies in our measuring methods and/or equipment. Alternatively, the results may point to an uneven distribution of pruinescent wax layers in areas of dark and bright coloration, or to differences in the density of wing venation. However, there is no indication yet for either hypothesis. Further studies should include scanning electron microscopy of the wing surface. Less delicate and more robust species (e.g. *Libellula*), will potentially exclude weighing and measuring artifacts. Sample sizes should be increased.

References

1. Kuhn, W. R. (2016). Three approaches to automating taxonomy, with emphasis on the Odonata (dragonflies and damselflies) (dissertation). Rutgers University, Newark, NJ.
2. Baskinger, G. M., J. L. Ware, D. D. Kornell, M. L. May, and K. M. Kjer. (2008). A phylogeny of *Celithemis* inferred from mitochondrial and nuclear DNA sequence data and morphology (Anisoptera: Libellulidae). *Odonatologica* 37(2) 101-109.
3. GIMP 2.10.6, www.gimp.org (1997-2018), retrieved on August 24, 2018.
4. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
5. Suhling, F., Sahlén, G., Gorb, S., Kalkman, V.J., Dijkstra, K.D.B., van Tol, J. Order Odonata (2015) Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates, pp. 893-932. Cited 1 time.
6. Pilgrim, E.M. and Von Dohlen, C.D., (2008). Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. *Systematic Entomology*, *33*(1), pp.159-174.