Title

Body Size and Taxonomic Influence on BeeWing-Vein Density

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Abstract

This study investigated bee forewing vein density as it relates to body size and taxonomic group. Within the entomological field of study, it is known anecdotally that wing venation is primarily conserved at the genus level more than any other taxonomic level. Using dorsal and slide-plated wing images, wing vein density (WVD) and intertegular span (ITS) was measured for bee species within different genera and families. A novel way of effectively measuring WVD was developed, a measurement that combines many previously used vein morphology characteristics. The study found that both taxonomic level and body size influence WVD, of which the taxonomic level of genus has the most significant effect, regardless of body size. Thus, this paper found that WVD can be useful for determining genus within a family and gives further insight into insect wing vein evolution.

Introduction

All winged insects have wing veins that form unique patterns. A strong correlation between wing size and body size has been established in bees, which are found within the insect order Hymenoptera (Bullock 1999). The number of wing veins present is also known to increase with body size in the case of Hymenoptera (Danforth 1989). However, how this pattern relates to the density of veins observed in the wing has not previously been studied and the overall relationship between wing-vein number and body size has not been evaluated in bees in the absence of other hymenopteran taxa.

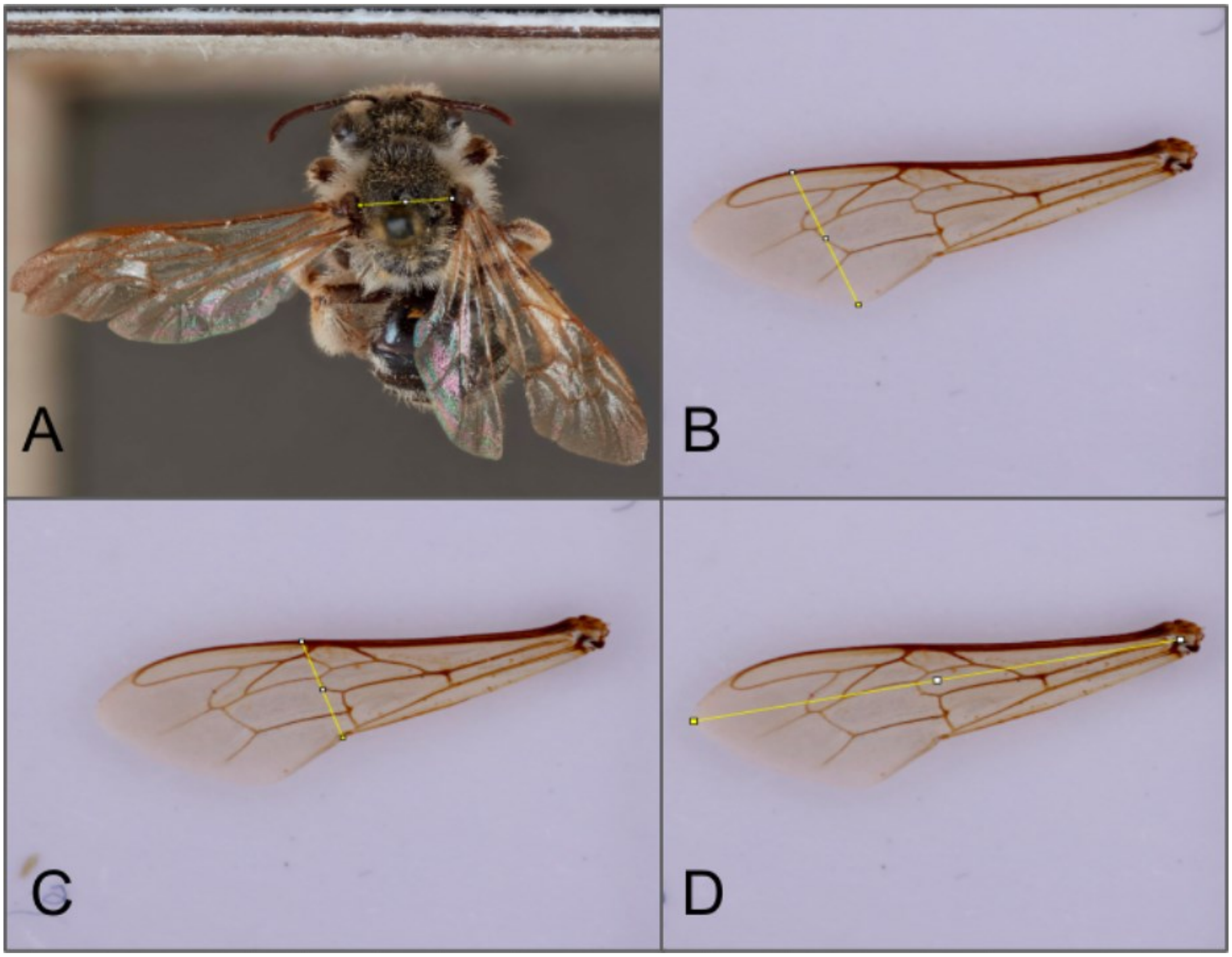
The functions of insect wing veins include structural integrity, hemolymph circulation, crack-propagation resistance, wing corrugation development, sensory structure support, and passive wing deformation for flight stability (Hoffmann et al. 2018). Vein pattern in bees is also an important trait for identification at the genus level. Historically, determination of a specimen’s genus has been done through the use of standard field classification guides that allow for classification based on traits visible to the human eye. These guides do not offer instructions for classification down to the species level because the variation between species of the same genus is difficult to distinguish and often requires a microscope as well as an expert in this field of study. The species-identification experts use wing morphology, along with other physical traits such as color, stripes, tongue, and antennae (Hall 2011). However, it has been shown that consideration of wing morphology alone is sufficient for both genus and species level identification (Steinhage et al. 2006). The automatic system that Steinhage et al. implement to identify species utilizes, among other aspects, wing-vein-length ratios, vein-junction distances, and vein-cell-area ratios. However, since the density of the veins is likely strongly dependent upon those wing vein aspects, this one value of vein density can be utilized for identification at the genus level.

The objective of this project is to determine if wing-vein density (WVD) is more strongly correlated with body size or taxonomic group. In addition, the production of a consistent and efficient method of measuring WVD is necessary. Beyond anecdotal evidence that wing venation is primarily conserved at the genus level, this project aims to demonstrate that concept statistically. By utilizing the images of bee specimens taken as part of the Big Bee Project (http://big-bee.net), further insight can be gained regarding the impact body size has on the WVD of a bee, as well as how much of it is influenced by taxonomic level, regardless of body size.

Methods

48 female specimens from 29 species (five families, 17 different genera) were measured for WVD, body size, forewing size, and forewing width. The pinned bees were analyzed for intact right forewings and two specimens per species were imaged dorsally using a 100mm Canon Macro lens top-down setup. Species were selected based on the total number of that species available in the UCSB Invertebrate Zoology Collection as well as the inclusion of a broad range of families and genera. The intertegular span (ITS) was measured from images to determine body size. The right forewing was removed, put between two dry microscope slides, and photographed.

Fig. 1 Measurements using ImageJ **–** A: Intertegular Span, B: Distal Wing Width, C: Proximal Wing Width, D: Wing Length.



Additional specimens were obtained through the Bee Library online bee and trait-image portal (https://library.big-bee.net). The specimens in this database allowed for more options in taxonomic variation. However, the database did not include images of separated wings but rather close-ups of the wings still attached to the specimen. 13 out of the 48 specimens measured were done so using images from the database.

The images were cleaned up in Adobe Photoshop and two wing images were created. Using the natural contrast between veins and the surrounding thin membrane, one image was created with just the veins thresholded in black and another image with the entire wing fully blacked out. ITS, wing width, and wing length were measured by images using ImageJ (Fig. 1). ITS is known to be an approximate calculation for dry body weight in bees (Cane, 1987). The wing area was measured using the blacked-out version of the wing and the vein area was measured using the vein-thresholded version (Fig. 2). WVD was measured as the ratio between the area of the veins to the area of the entire wing.

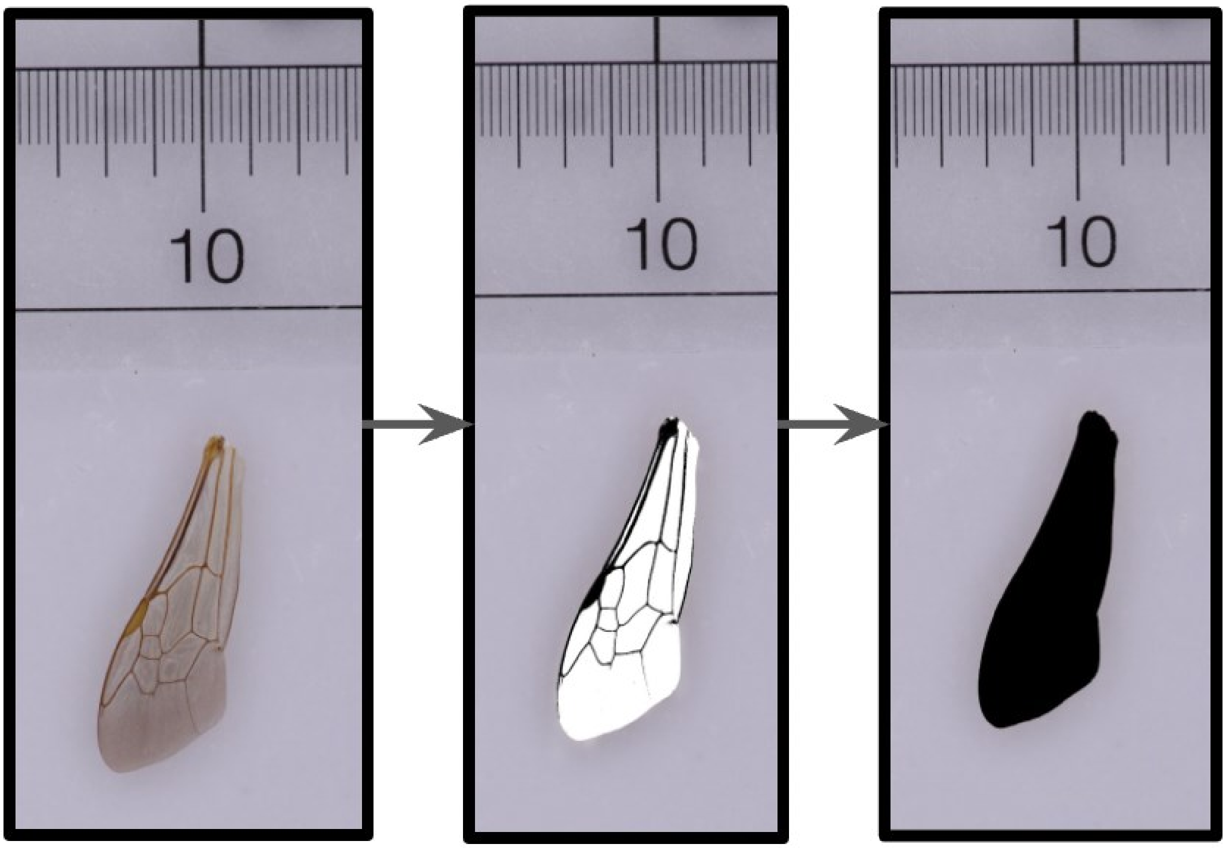


Fig. 2 (Left) Original image, (Middle) Contrast-thresholded veins, (Right) Whole wing blacked out.

The Pearson Correlation Coefficient was used to determine the relationship between WVD and ITS. One-way and two-way ANOVA tests were used to examine WWD for each taxonomic level (family, genus, species). These tests were used to determine the relationship between taxonomic level and WVD, as well as which taxonomic level had the strongest influence, both when accounting for and not accounting for body size. All analyses were conducted in the programming language "R" after confirming random variance (linear model and residual plot) and a normal distribution of the data (Q-Q plot and Shapiro-Wilk Test).

Results

The Pearson Correlation Coefficient resulted in a moderate positive correlation between WVD and ITS (R-value = 0.431; p-value = 0.00226). As shown in Figure 3, as ITS increases, the WVD also increases. The coefficient of determination (R^2 value) was 0.1853, indicating that the data was scattered in relation to a linear relationship between WVD and ITS.

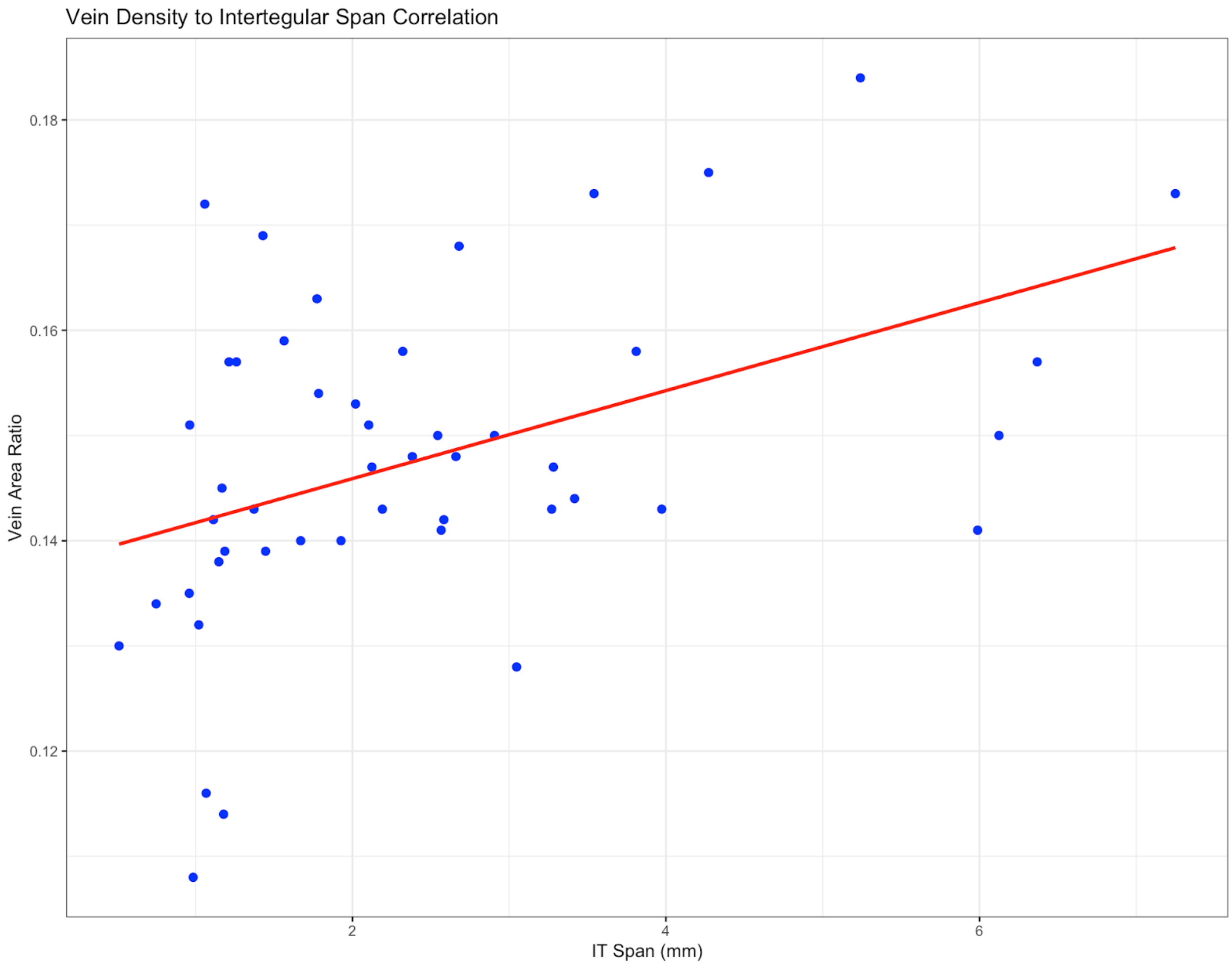


Fig. 3 Scatter plot with linear regression showing the correlation between wing-vein density and body size.

When determining the statistical significance of the relationships between species, genus, and family to WVD regardless of ITS, one-way ANOVA tests showed p-values of 0.0131, 3.12×10^{-5}, and 0.00845, respectively. Accounting for body size by conducting two-way ANOVA tests, however, the tests showed p-values of the relationships between species, genus, and family to WVD of 0.0136, 1.68×10^{-5}, and 0.00507, respectively. In addition, the p-values reported for ITS in relation to taxonomic group and WVD were 0.26, 0.0647, and 0.02368, with respect to species, genus, and family (Fig. 6).

WVD was plotted against each genus and each family studied. The family *Apidae* had the most specimens with the highest WVD overall and the family *Andrenidae* had the most specimens with the lowest WVD overall (Fig. 4). All of the interquartile ranges of each family overlap each other significantly. The outlier, *Megachilidae*, is not significant since that family only included one specimen. In terms of genus, *Xylocopa* had the highest WVD with a ratio of vein area to wing area of around 0.175, and *Perdita* had the lowest WVD with a ratio of around 0.11 (Fig. 5).

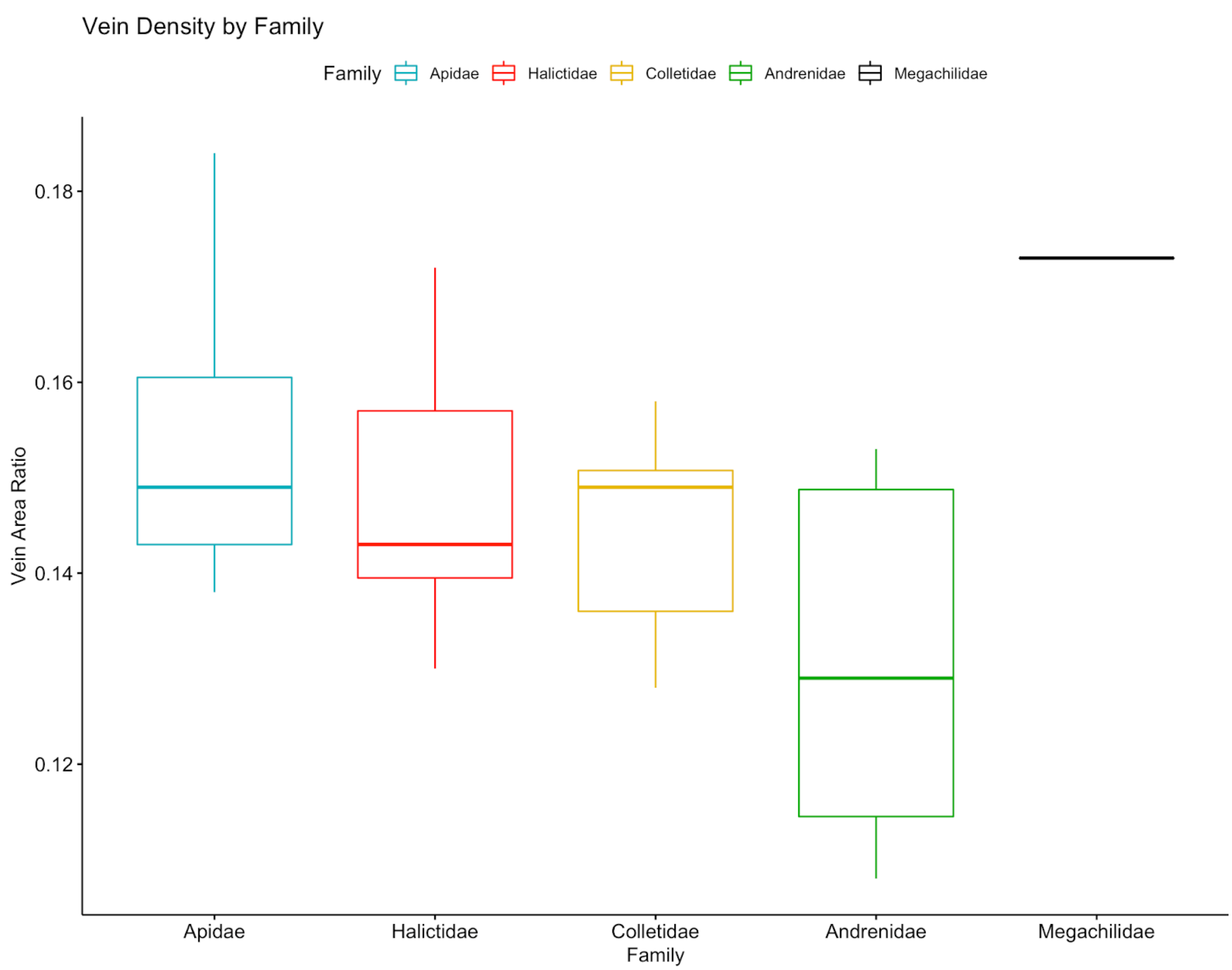


Fig. 4 Boxplot showing overall wing vein-density ratio measured per family: Apidae (n=16), Halictidae (n=19), Colletidae (n=6), Andrenidae (n=6), Megachilidae (n=1).

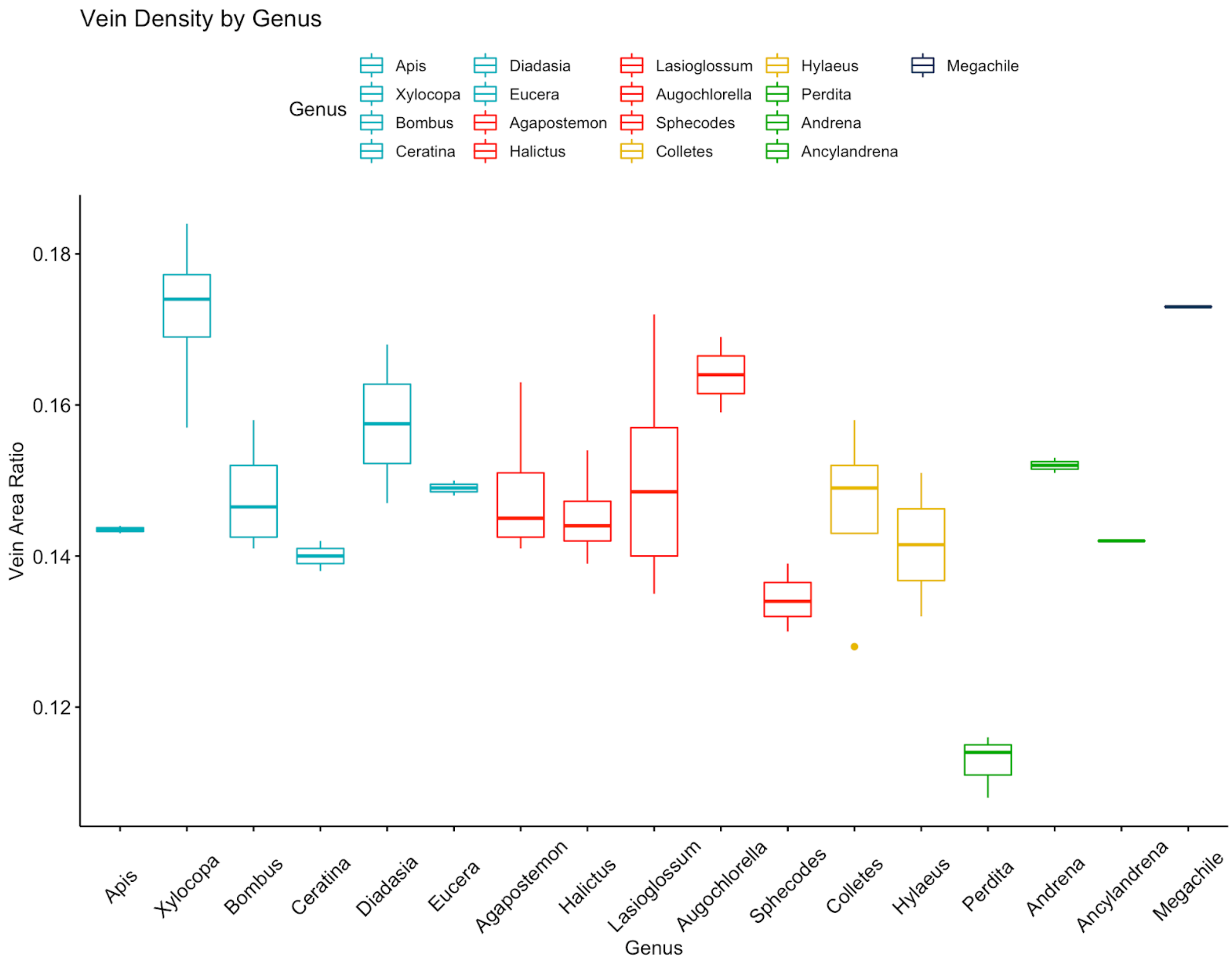


Fig. 5 Boxplot showing overall wing-vein density ratio measured per genus: Apis (n=2), Xylocopa (n=4), Bombus (n=4), Ceratina (n=2), Diadasia (n=2), Eucera (n=2), Agapostemon (n=4), Halictus (n=4), Lasioglossum (n=6), Augochlorella (n=2), Sphecodes (n=3), Colletes (n=4), Hylaeus (n=2), Perdita (n=3), Andrena (n=2), Ancylandrena (n=1), Megachile (n=1). Refer to figure 4 for the color key by family.

P-value of ___ on density:	Species	Genus	Family	ITS
Species	0.0131			
Genus		3.12E-05		
Family			0.00845	
Species + ITS	0.0136			0.26
Genus + ITS		1.68E-05		0.0647
Family + ITS			0.00507	0.02368

Fig. 6 Table showing the relative p-value from the one-way (Species, Genus, Family) and two-way (Species + ITS, Genus + ITS, Family + ITS) ANOVA tests as it relates to the WVD.

Discussion

The moderate positive correlation between WVD and ITS indicates that bee body size is a factor that influences the density of bee wing veins. Even though the Pearson Correlation Coefficient test resulted in a statistically significant p-value, the coefficient of determination was low indicating that there are additional factors besides body size that influence the WVD.

Our results indicate that wing-vein density is conserved at the genus level and that the genus in which a bee is found determines WVD more than its designated family. The results show that all three taxonomic levels have a statistically significant relationship to WVD, with the genus having by far the most and family having the least. It should be noted, however, that there was more data at the genus level than family, and even fewer for species. There were only one to two specimens of the same species included in this study, which should be taken into account when interpreting the p-value of species as it relates to WVD.

Looking at both body size and taxonomy together, it appears that they both have some level of significance in determining WVD. Nonetheless, the results indicate that the genus has a much greater impact on predicting WVD than body size alone. There are a few examples illustrating this point in the dataset. When looking between genera in the family Apidae, the WVD differs quite significantly and the genus with the highest WVD, *Xylocopa*, is also a genus of relatively large bees. Yet, another genus also within Apidae, *Diadasia*, also had a high WVD but is actually less than half the ITS of the *Xylocopa*. Furthermore, the genera *Sphecodes* and *Hylaeus* both had smaller ITS than the genus *Perdita*, but *Perdita* ended up having the lowest WVD by far.

The results and findings of this study increase our understanding of the relationships between WVD, body size, and taxonomic group. It was noticed that all bee-wing venation is very similarly conserved in the genome as most vein patterns followed analogous designs. In addition, all of the specimens measured fell within a small range of WVD to one another. This suggests that there is an ideal amount of wing venation necessary for the most efficient flight, which is crucial since flying is the most energy-consuming activity a bee must conduct (Kammer and Heinrich 1978). Since wing veins serve mainly as a form of structural support, having a high WVD is beneficial for good wing integrity over time. However, the main physical barriers to a higher WVD seem to be the need for passive wing flexion and the requirement of low wing weight. This should give a better understanding of why WVD is conserved so well among different species of different families of bees, but the patterns that those veins create are not conserved nearly as much.

Further data collection would be beneficial to reduce the weight that any one taxonomic group has on the overall relationships. The other measurements taken in this study such as wing length and width could prove to be an essential aspect of understanding wing vein morphology and density. This study aimed to include at least two specimens of each species but that goal proved to be more challenging than anticipated. Likewise, this study also aimed to have an equal number of specimens per genus and family but that became a difficult aim to maintain. Having access to female bee specimens with intact wings was the main cause of not reaching those goals. However, given more time and resources, further research should be conducted in order to include a larger variety and number of genera and species, as well as a wider range of body sizes.

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