Geometric Morphometrics Captures Variations in Wing Development of Black Soldier Flies

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Research Article

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Geometric Morphometrics Captures Variations in Wing Development of Black Soldier Flies

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Abstract

The black soldier fly is considered an important insect due to its ability to convert organic waste into high quality organic fertilizer. Studies have shown that food quality at the larvae stage contributes greatly to their development. In this study, we applied geometric morphometric measurements to assess the variations in the development of the black soldier fly fed on different food substrates as captured in the wing. Eggs of black soldier fly were collected from the field and cultured on different food substrate such as; restaurant waste, fruit waste, wheat bran and layer meal until maturity. The right fore wings of 140 individuals of black soldier fly were used for the experiment. The results showed a significant difference between the shape of landmarks within each food substrate group. Landmarks 3, 7 and 9, corresponding to end of first radial vein R_1, intersession between medio- cubital vein and cubito- anal vein (m-cu and CuA), and intersession between cubito Anal vein and Anal vein (CuA + CuP) contributed most to the variation between the different food substrates. Partial least square showed a strong association between food substrate and wing development. Therefore, the variations in wing development could be due to the impact of nutritional compositions quality of the different food substrates.

1.0. Introduction

The Black soldier fly (BSF), *Hermetia illucens*, L. (Diptera: Stratiomyidae) is one of the important insects in entomophagy and perhaps the best insect feed products in the EU [1-5]. Its larvae is proposed as a promising technology for treating organic wastes or livestock manure [6-8]. Black soldier fly larvae are voracious feeders of decomposing organic materials and has the capacity to reduce these wastes by 42 – 70 %, [8-10]. This convert it into high quality organic fertilizer, which restores soil fertility. The larvae also reduce potential pollutants such as organic chemicals in manure and cause significant reduction of *E. coli* and *Salmonella enterica* in chicken and cattle manure [11,12]. The larval biomass contain around 42% protein and 35% fat [13], based on dry matter, including favourable profiles of essential amino acids 13], which are higher compared to every other plant-and animal-based food [14]. These properties make BSFL a suitable feed supplement candidate for fish and various livestock feed production [10, 15].

Most of the studies on black soldier fly, (*Hermetia illucens*), have focused on its high rate of conversion of biomass to protein and lipid, and its potential role in waste management [16]. Globally, little is known about geometric morphometric assessment on the variation of wing development of black soldier flies fed on different food substrates. The development of some features such as the wing of the black soldier fly have been shown to vary [17], depending on the quantity and quality of the diet supplied to them [18]. Geometric analysis of the size of the wings is a widely used method for studying the effect larval diet quality on the growth of Diptera [19].

Morphometric analysis is a technique which measures shape by Cartesian landmark and semi landmark coordinates, which has the potential of distinguishing morphological shape variables. This technique was applied to study the variability in size of laboratory strains of flies and variations among natural inhabitants [20]. Wing size variation assessment (based on centroid size analysis) provide valid and reliable statistical information [21]. Geometric morphometrics (GM) also indicates phenotypic variability and population structure with less effort [22], despite, its quality for wing assessment, studies on *H. illucens* species are still limited [23-24]. In GM, both wing size and shape are assessed autonomously for heritability and environmental sensitivity [25]. Variances from size and shape show consistent information about genetic variation of natural populations showing high and stable heritability [26-29].

Morphometric characters may indicate reliable information concerning the biology of insects, due to the influence of genetic and environmental conditions. Studies on insect wing geometry could provide significant information for species identification and description of population diversity. Geometric morphometric analyses utilize the wings due to its ability to provide well-defined landmarks for morphometrics and the metric properties for precise quantitative information. Landmarks are the intersections that biological structures are sampled. These points provide particular geometric description of the modifications in shape of a structure. A number of entomologists have carried out commendable works on insect wing morphometrics as well as in mosquitoes. The current research is therefore based on the hypothesis that different larvae substrates can contribute to the variability in adult black soldier's wing vein development. This study was conducted to investigate the variation in the wing development of black soldier flies fed on different substrates through landmark-based geometric morphometric analysis. The findings of this study can provide insight into the population structure, ecology, and taxonomic identity of the black soldier fly. Considering the importance of black soldier fly wings in insect behaviour and physiology, the differences could also provide useful information on the quality of diet, distribution, and variability in the wing development based on the food substrate they were fed on.

2.0. Results

Results obtained from all the analyses showed significant differences ($P< 0.0001$) between wing characters of black soldiers fly larval development fed on different food substrates. Further, three wing veins showed significant variation in black soldier fly populations that were fed on different food substrates. These veins were the following; end of first radial vein R_1, intersession between medio- cubital vein and cubito- anal vein (m-cu and CuA), and intersession between cubito Anal vein and Anal vein (CuA + CuP). Procrustes ANOVA showed a significant difference between the shapes of the landmarks ($P = 0.0001$) and the centroid size

($P < 0.0001$) of the landmarks within groups of black soldier flies fed on different food substrates (Table 1).

Table 1: The table below shows ANOVA values for the centroid and shape of landmarks.

Centroid Size					
Effect	SS	MS	Df	F	*P*-(param.)
Individual	461497 326867	153832.442289	3	8.33	< 0.0001
Residual	2420517.234600	18477.230798	131		
Shape					
Individual	0.01855603	0.0003436302	54	2.57	0.0001
Residual	0.31537027	0.0001337448	2358		

During the principal component analyses, it was found that landmark 3 contributed most to the variability within the population followed by landmarks 7 and 9. (Figure 1). These landmarks correspond to the following wing features; end of first radial vein R_1, intersession between medio- cubital vein and cubito- anal vein (m-cu and CuA), and intersession between cubito Anal vein and Anal vien (CuA + CuP). A sum of the Eigen score values of the first three components (PC1, PC2, PC3) contributed 66.94% of the total variability with each contributing 29.711%, 21.235 and 16.001% respectively (Figure 2).

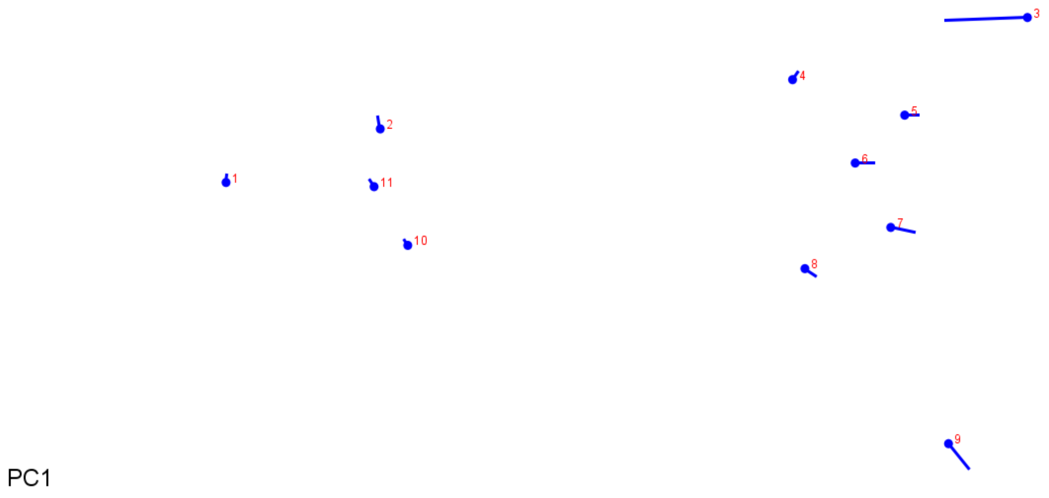


Figure 1: This shows the shape changes or deviations from the centroid for each landmark.

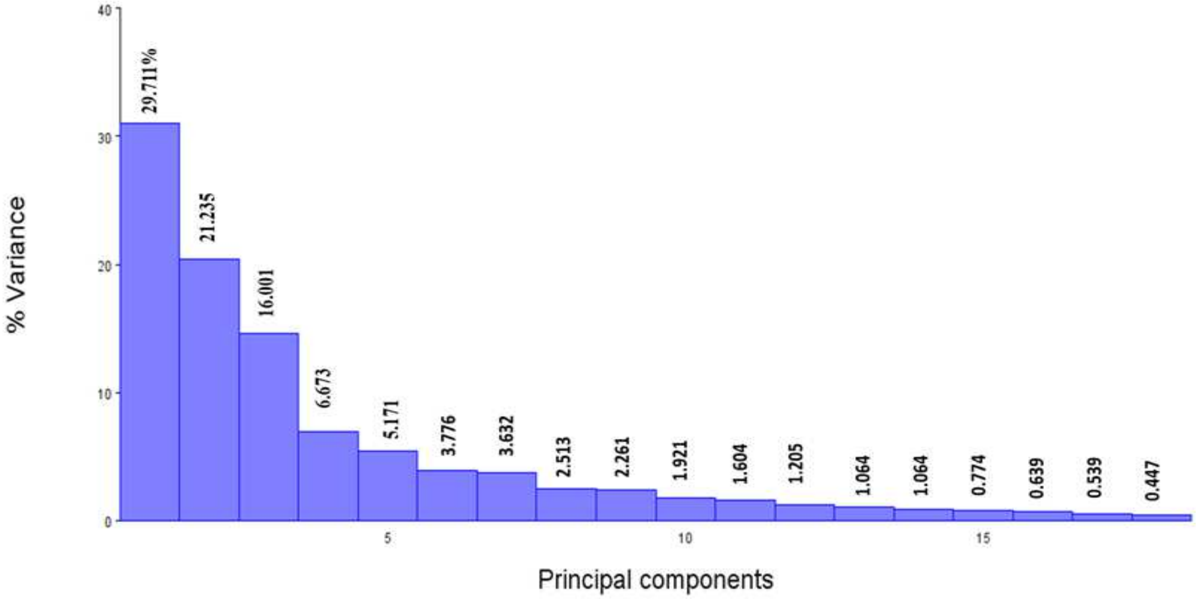


Figure 2: This shows the eigen values for each component.

Discriminant Function Analyses (DFA) showed significant comparative differences within the populations of black soldier fly fed on different food substrates. Wing veins representing landmarks 3, 4 and 9 showed greater deviation from the centroid in all analyses except layer meal and restaurant waste where landmark 7 also showed marked variation from the centroid. In the black soldier fly populations that were fed on fruit waste and restaurant waste, significant differences ($P = 0.0253$) were observed with 79.41% of the population were found to be distinct with 55% accuracy in cross validation tests. On the other hand, DFA score for populations fed on restaurant waste were 82% discriminated with 62% accuracy in cross validation (Figures 3a & 4a). Further to this, a nonsignificant difference ($P = 0.1499$) was observed between populations fed on fruit waste and layer meal where DFA scores of 82% and 76% respectively with cross validation of 62% and 61% respectively. (Figure 3b & 4b). Discriminant function analysis between samples fed on fruit waste and wheat bran showed non - significant differences ($P = 0.0624$), with DFA scores of 76% and 82% respectively while cross validation was observed at 56% and 59% respectively (Figure 3c & 4c). Results obtained from DFA for samples fed on layer meal-restaurant waste, layer meal-wheat bran, restaurant waste-wheat bran showed significant differences within groups with respective *P* values of 0.0001, 0.0022 and 0.0017. The DFA scores for layer meal-restaurant waste were 90% and 94% with cross validation accuracy of 67 % and 65 % respectively, while scores between layer meal-wheat bran 85% in each group with cross validation of 67% and 76% respectively. Further, results obtained for DFA score discriminated restaurant fed sample by 85%, similar for samples fed by wheat bran with cross validation of 71% and 62% respectively (Figures 3d-f, 4d-f).

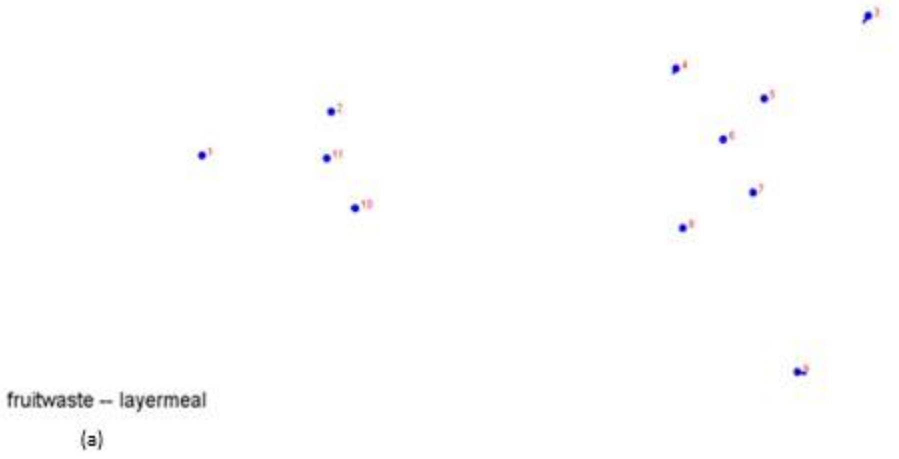


Figure 3a: Graphical representation of shape difference for discriminant function analyses between samples fed on fruit waste and layer meal

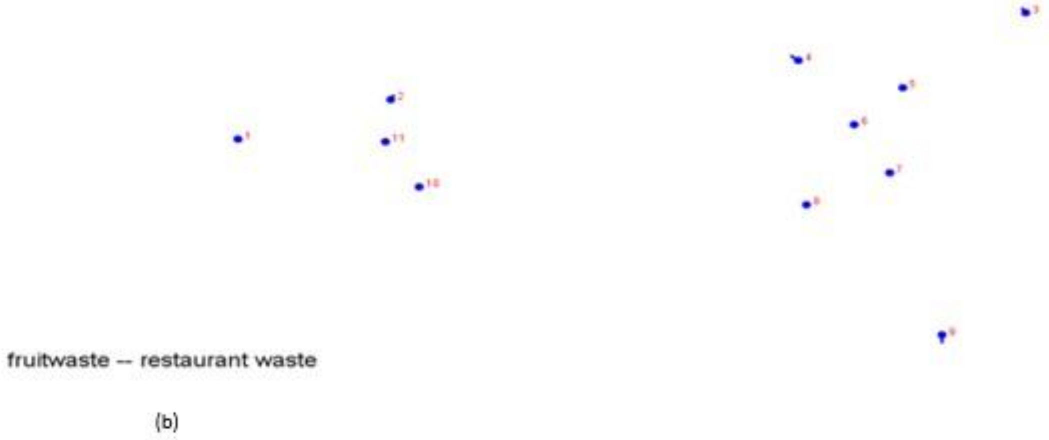


Figure 3b: Graphical representation of shape difference for discriminant function analyses between samples fed on fruit waste and restaurant waste

Figure 3c: Graphical representation of shape difference for discriminant function analyses between samples fed on fruit waste and wheat bran

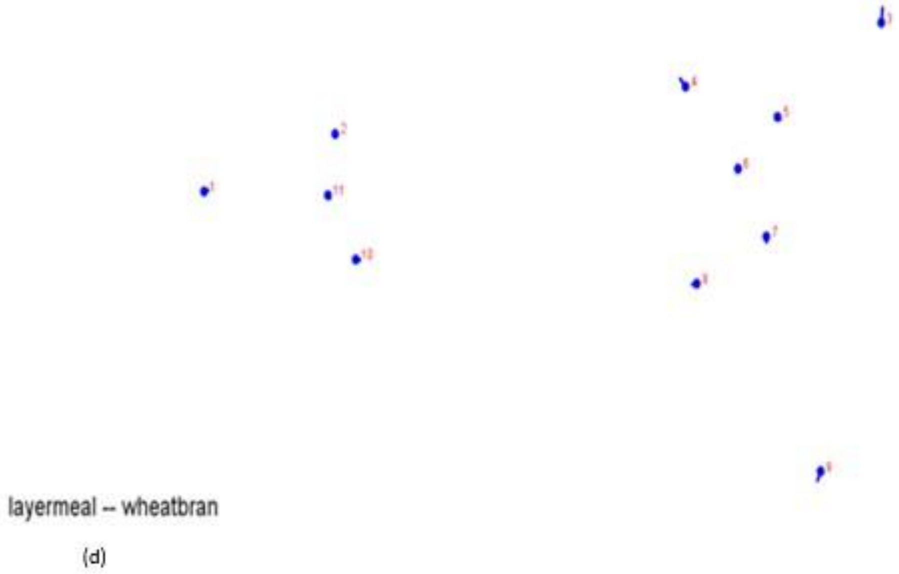


Figure 3d: Graphical representation of shape difference for discriminant function analyses between samples fed on layer meal and wheat bran

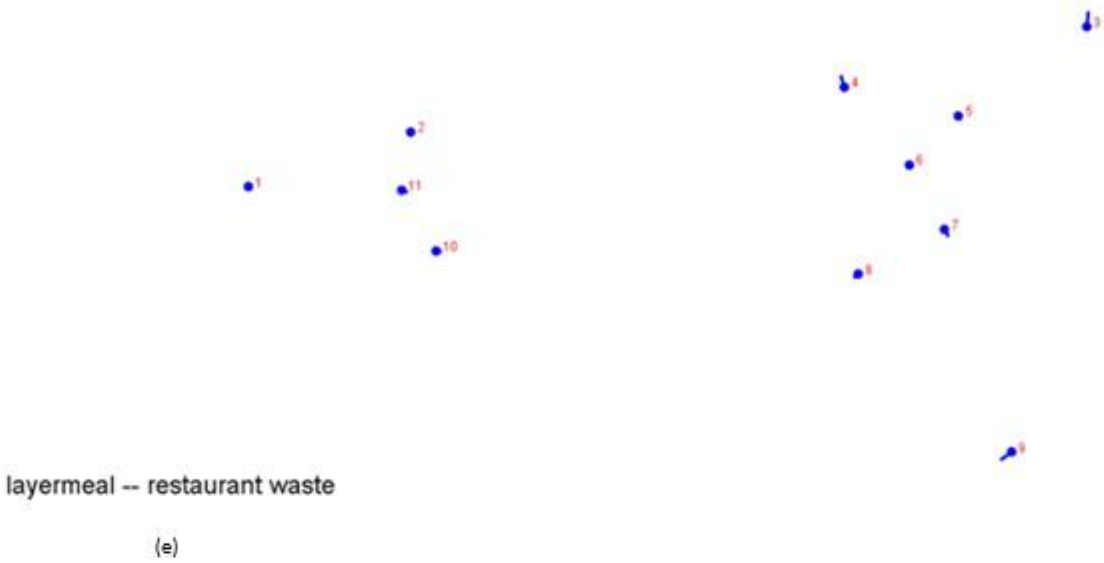


Figure 3e: Graphical representation of shape difference for discriminant function analyses between samples fed on layer meal and restaurant waste

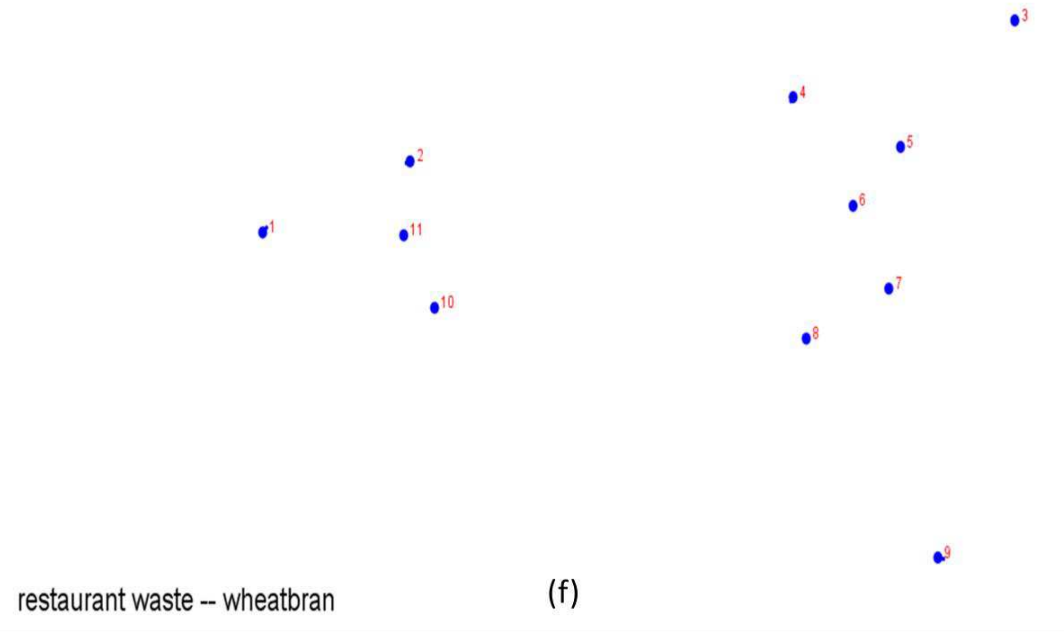


Figure 3f: Graphical representation of shape difference for discriminant function analyses between samples fed on restaurant waste and wheat bran

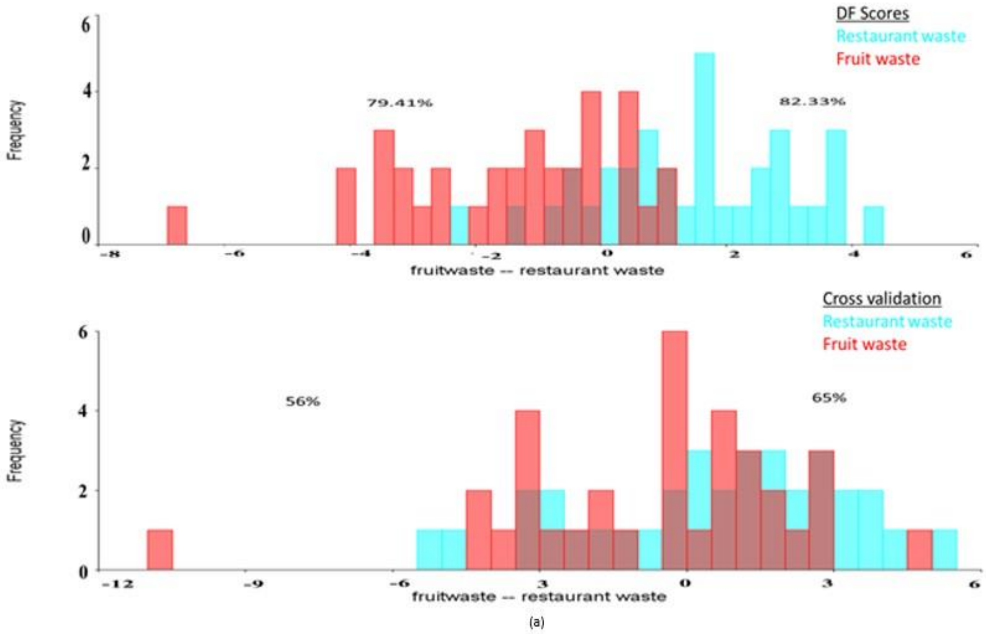


Figure 4a: Graphical representation of discriminant function scores (a) and cross validation scores (b) for fruit waste – restaurant waste

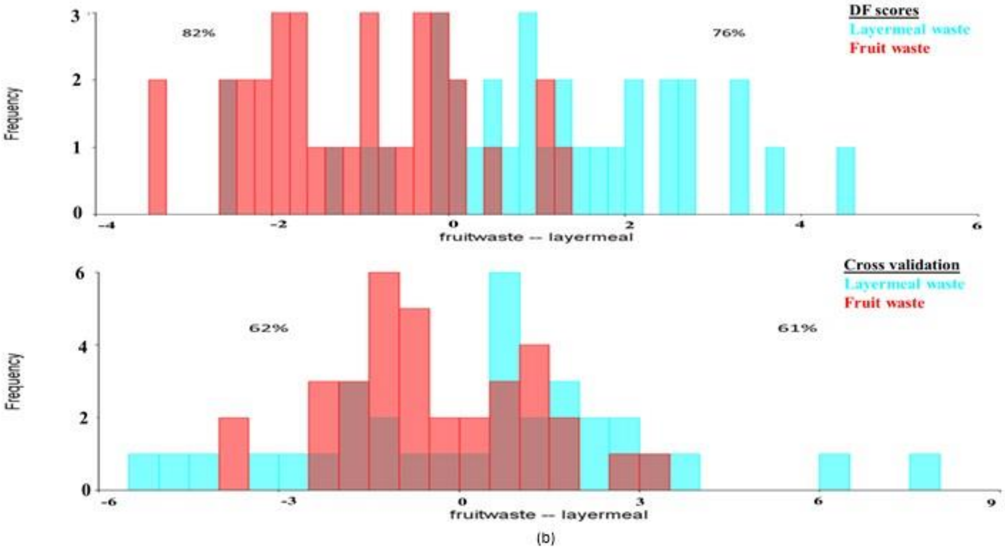


Figure 4b: Graphical representation of discriminant function scores (a) and cross validation scores (b) for fruit waste –layer meal

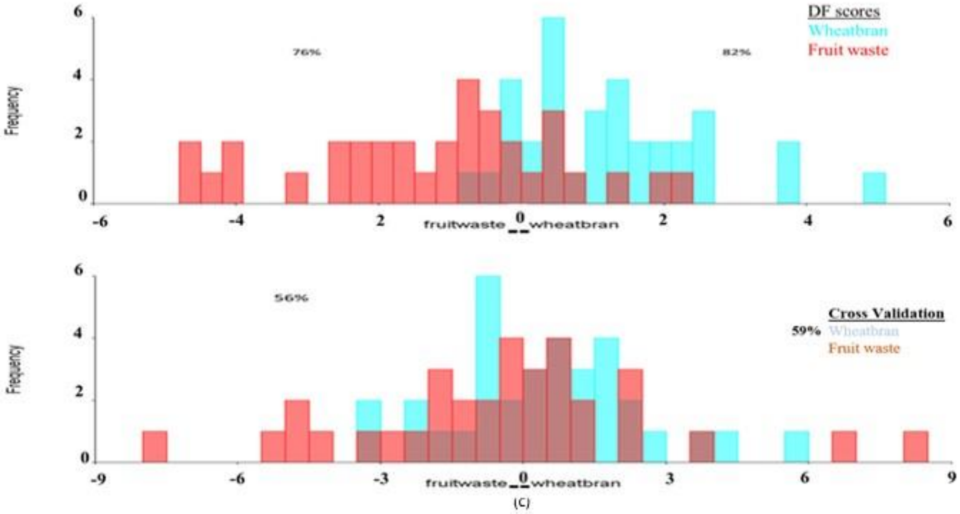


Figure 4c: Graphical representation of discriminant function scores (a) and cross validation scores (b) for fruit waste – wheat bran

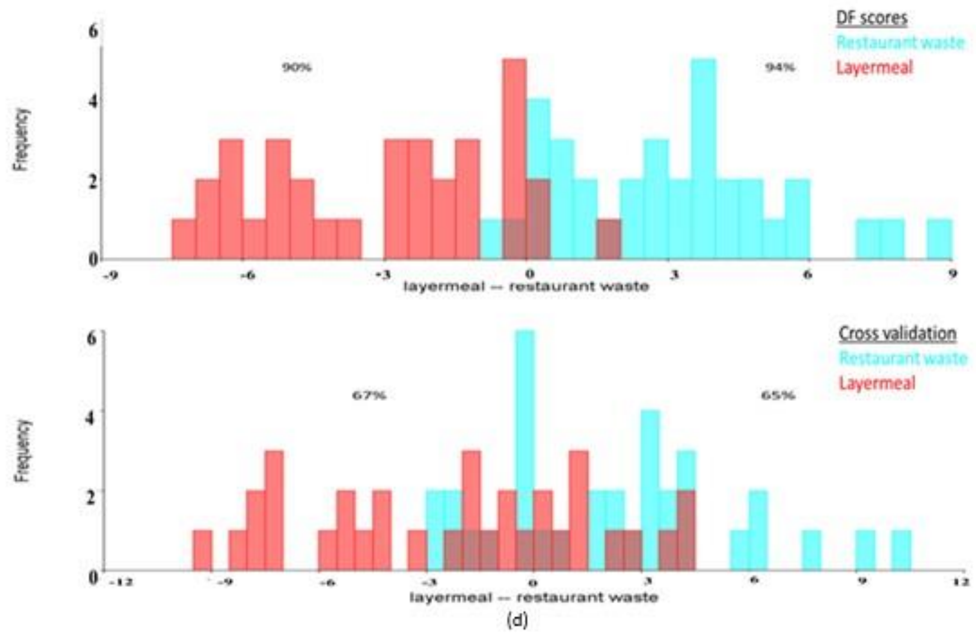


Figure 4d: Graphical representation of discriminant function scores (a) and cross validation scores (b) for layer meal – restaurant waste

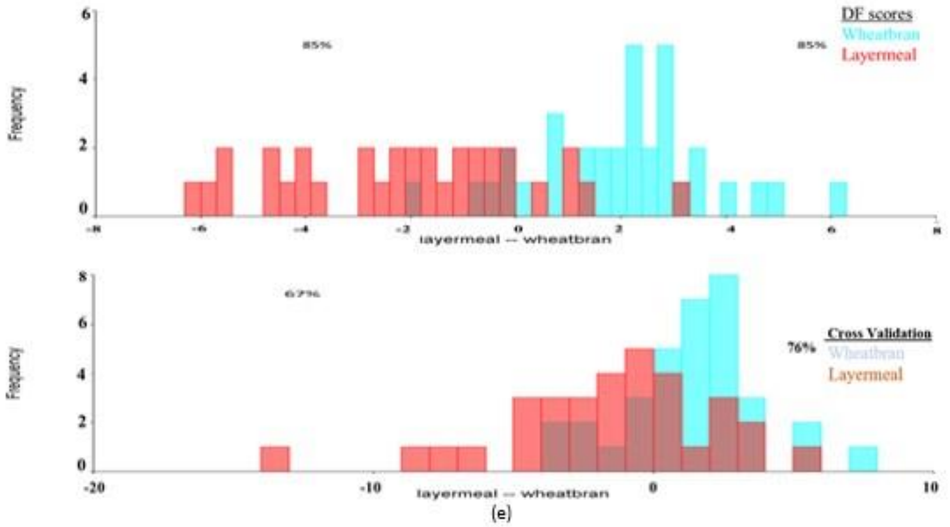


Figure 4e: Graphical representation of discriminant function scores (a) and cross validation scores (b) layer meal -wheat bran

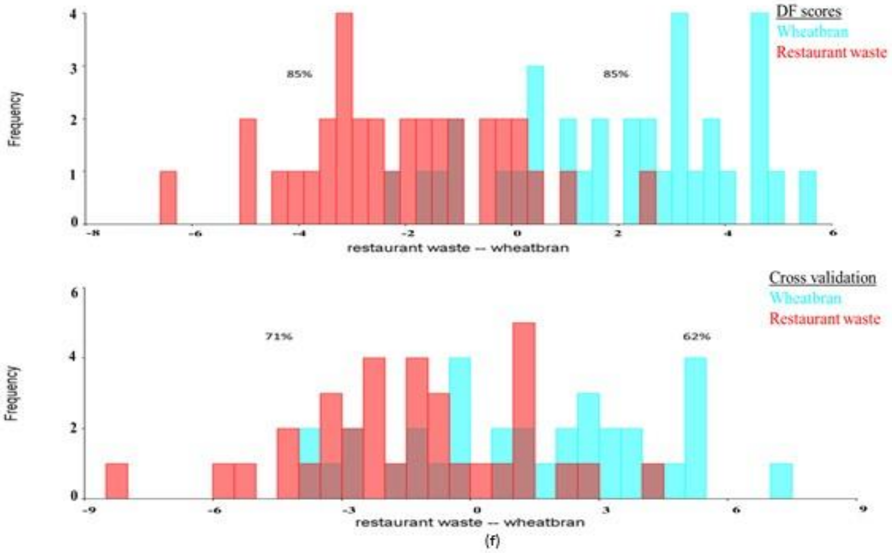
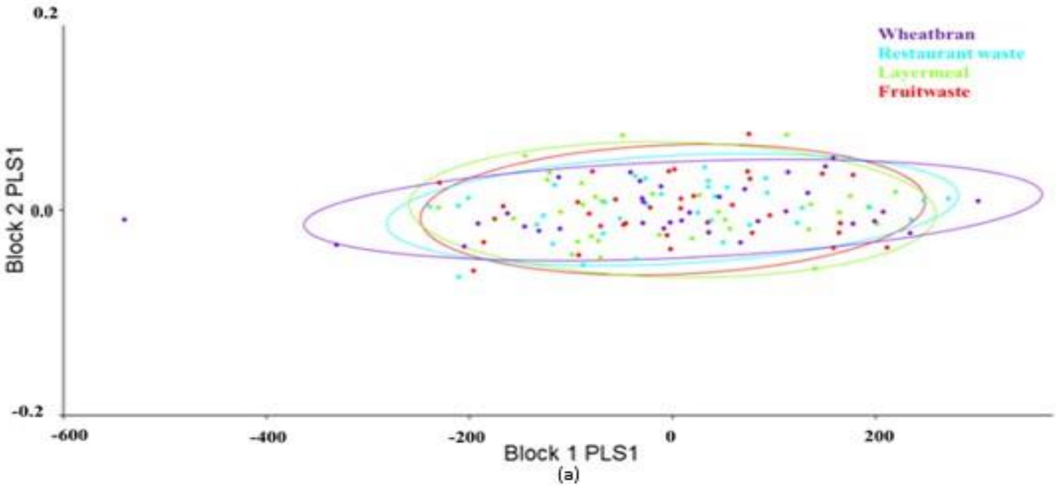


Figure 4f: Graphical representation of discriminant function scores (a) and cross validation scores (b) restaurant waste – wheat bran

Graphical representation of partial least squares showed a strong association between black soldier fly feed substrate and wing feature development with overall strength of association between feed substrate having RV coefficient of 0.0224. Further, populations fed on wheat bran showed the highest variability within wing characters. In figures 5a, the ellipse for wheat bran population further spread from the centroid on across the latitudinal axis as compared to populations fed on other food substrates. Samples fed on restaurant waste were also observed to show variation around the centroid, with fruit waste and layer meal exhibiting almost the same extent of variability with their respective groups (Figure 5a, 5b).



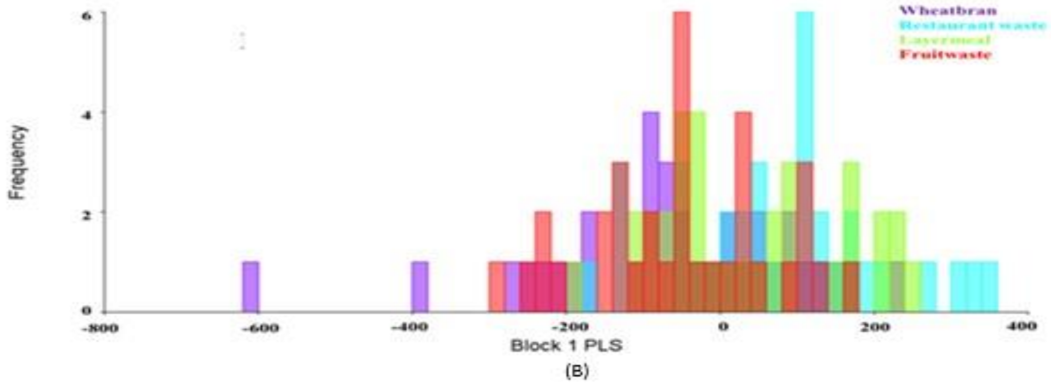


Figure 5 a & b: Graphical representations of partial least squares showing association among food substrates and wing character variability.

3.0. Discussions

The development of insect features including wing features is attributed to their food resources and genetic traits [30]. In black soldier flies, wing developments vary, depending on the quantity and quality of the food supplied to them at the larval stage [17-18]. In the present study the results showed three main wing features that were significantly affected when BSF populations fed on the different food substrate. Generally, in insect wing vein development, the presence of additional or incomplete veins between fore wing cells occur around the marginal and cubital wing cells. In this present study, three main wing veins and vein intersections were found to be variable between cubital and medial veins. These variations in veins could therefore be due to (high sensitivity in response to the quality of nutritional compositions of the different food substrates. The present study also showed significant variations between the type of food substrate and the wing vein characteristics of BSF. Previous reports indicate that organic wastes release several volatiles such as; alcohol, aldehydes, and organic acids during decomposition that attract insects in response to variable developmental needs [31-33].

This suggest that these significant variations within the wing vain features could possibly be due to the presence of some volatile compositions in the substrates. However, it appears that the contribution of each food substrate to the variability is greatly dependent on the amount of volatile compositions present. Larvae that are cultured for waste treatment and feed production, are reared on wastes such as; vegetable, fruit, and other market and restaurant waste, with the aim of reducing cost of colony maintenance [33]. In this present study discriminant assessments showed BSF samples fed on layer meal-restaurant waste, layer meal-wheat bran, and restaurant waste-wheat bran showed significant differences within groups. However, no significant differences were observed within groups that fed on fruit waste- layer meal, fruit waste-restaurant waste and fruit waste -wheat bran. This possibly implies that in wing vein development of BSF, earlier sets of food substrate may induce significant growth differences, compared with the latter sets of food substrates. This result will be useful in choosing best feed substrate for any breeding experiment.

Poor nutrition may stimulate the production of alatae by the aphid *Myzus persicae*, however, “low energy” diets do not support physiological conditions necessary for wing development [34]. Suboptimal nutrition is often thought to stimulate wing formation in aphids by influencing the “milieu intérieur” in a way that stimulates the process of wing determination. Though the nutritional compositions of each feed substrate were not tested, populations fed on wheat bran showed the highest variability or dispersion with restaurant waste fed populations in close trend. This indicates that, comparatively, wheat bran contains high energy compositions, which stimulated wing vein development by influencing the physiology of the of the black soldier fly. The cross-talk of bombyxin and 20-hydroxyecdysone are reported to stimulate cell division and growth of wing imaginal disks [35]. Moreover, the level of bombyxin in insects’ hemolymph is modulated by the brain in response to variation in nutrition, as well as the mechanism that coordinates the growth of internal organs with overall somatic growth. The contribution of high-level energy substrate to variability in wing vein development has been reported in *Drosophila suzukii* on carbohydrate-rich media [36]. It is reported that adult flies showed a strong preference for carbohydrate-rich foods in response to oviposition and in transpecific interactions, however, larval survival and eclosion rate were strongly dependent on protein rich food substrates.

4.0. Conclusions

The results of this study show that three wing veins seem to show significant variation in black soldier fly populations that were fed on different food substrates. These veins include the following; end of first radial vein R_1, intersession between medio- cubital vein and cubito- anal vein (m-cu and CuA), and intersession between cubito Anal vein and Anal vein (CuA + CuP).
Moreover, there was a strong relationship between the different food substrates and BSF wing vein developments. Black soldier fly samples that were fed on layer meal-restaurant waste, layer meal-wheat bran, restaurant waste-wheat bran showed significant differences within groups. However, no significant differences were observed within groups that fed on fruit waste- layer meal, fruit waste -restaurant waste and fruit waste -wheat bran.
Populations fed on wheat bran showed the highest variability or dispersion with restaurant waste fed populations in close trend, while fruit waste and layer meals showed low variations in populations around the centroid. Larvae from the same generation were used for these experiments, hence the results were not affected by genetic variation altering compatibility with diet. Our discovery could provide theoretical basis and a new perspective for studies on black soldier fly wing developments.

5.0. Materials and Methods

5.1. Rearing and sample collection

Larvae were obtained from a colony of black soldier fly, established from egg clutches trapped from the wild and breed in the laboratory over seven years at the Ghana Atomic Energy Commission, Accra, Ghana. Four groups of 100 individuals were selected and each group was fed on different feed substrates namely; restaurant waste, fruit waste, wheat bran and layer meal. The experiments were conducted in triplicate in plastic containers (Smart store classic 2, 21 x17 x11 cm) with netted lids, kept under ambient temperature of 28°C. In each box, 100 larvae (>0.2

cm in size, 5day old) were placed, giving a larval density of 0.6 larvae cm^2. Feed given was computed based on a formula by Ewusie et al., [33] as follows:

$$Feed\ amount\ (g) = \frac{Number\ of\ larvae \times 0.1\ g}{MC} \times 25 \times 70\% \tag{1}$$

Where MC = moisture content of diet
0.1 = Amount of feed per larvae per day [33]
25 = Estimated larval feeding duration (days) [33, 37].
70% = Adjusted moisture content of diets.

When the larvae reached the prepupal stage, they were removed with forceps into plastic pupation containers with sterilized sand as the pupation material till eclosion under ambient temperature and light. As soon as eclosion was seen in a container, the entire setup was placed in a medium-sized wooden adult cage and any dead adult was picked and transferred into bottles containing 70% alcohol solution for temporal storage till all eclosed adults died. The specimens were sent to the museum of the Department of Conservation Biology and Entomology, University of Cape Coast, Ghana, where extraction of wings, mounting of wings, capturing images of wings and morphometric assessments were done.

5.2. Geometric Morphometric Assessment

5.2.1. Slide Preparation

Specimens of approximately 55 adult black soldier flies were sampled from each of the food substrate groups. The right forewing of each adult (total 140 wings) was removed, placed between a microscope slides and secured in position with a microscope cover slip that had a drop of transparent nail polish carefully applied to the ends. Wings were relaxed in 30% alcohol before mounting unto slides. Each wing was photographed with a digital camera attached to a microscope. Wing images were captured and an image file was created in JPEG format. One TPS file was created from the image files using TPSUtil software (version 1.76; 38).

5.2.2. Wing Venation Characters

Eleven homologous landmarks were plotted at the junctions of the wing venation of the adult black soldier flies using tpsDig2 software version 2.31 [39]. One TPS file grouped all the eleven landmarks derived from each of the 140 wings of the black soldier flies fed on different food substrates (Figure 5).

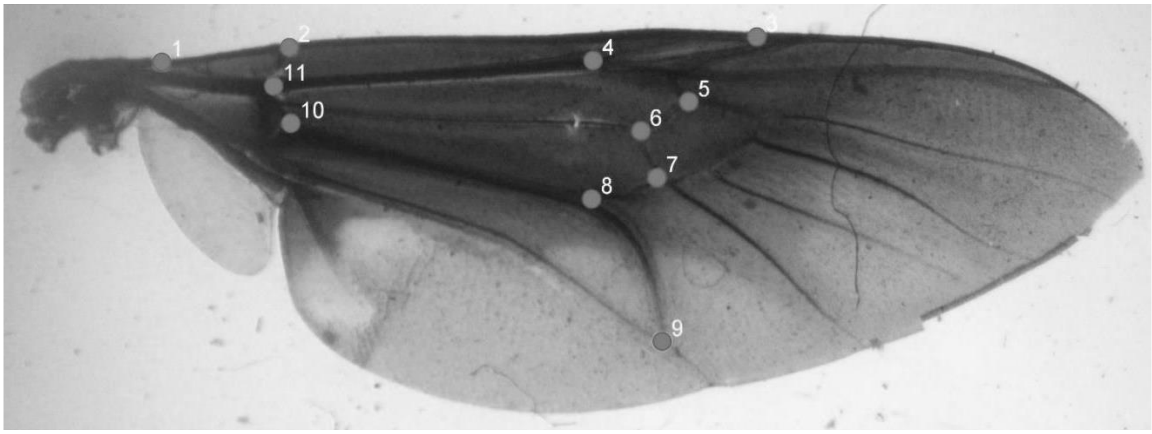


Figure 6: Forewing of black soldier fly with eleven homologous landmarks.

5.3. Statistical Analyses

The Procrustes coordinates of the 11 were first aligned using MorphoJ software (version 1.03) and existing shape changes within the different fly populations bred on different substrates were evaluated. Procrustes ANOVA was done to assess the relative amounts of variation among individuals, of asymmetry and measurement possible error. Further, Principal component analysis assessment provided the most significant features contributing to variability within the group. Discriminant function analysis was used to distinguish between groups of adults fed on different food substrates and this revealed the probability of correct and incorrect classification of samples for each food substrate in pairs [25, 40]. In this work, partial least squares (PLS) were used to examine patterns of Covariation between black soldier flies fed on different food substrates. In geometric morphometric, one or more of these sets contain shape data [25].

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