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Geometric Morphometrics of Geographic and Host-Associated Population Variations of *Bactrocera Invadens* in Uganda

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are among the most economically important pests of edible fruits worldwide. Because of the cryptic nature of most species in *Bactrocera* complex, it is not clear whether allopatric and host associated speciation has taken course among *B. invadens* populations in the different zones in Uganda. This would be important in the design of efficient management strategies. The morphological variation of 3 allopatric and host-associated populations of *B. invadens* was studied using Euclidean distance matrix analysis and variance structure coordinate system geometric morphometrics techniques. Size and shape were analyzed throughout 15 landmarks for 360 specimens after symmetrization process. Host type significantly affected wing size and shape (P = 0.0001), with CVA showing host population discrimination with very high degree of population classification (95.03%) accuracy. Mango wings were significantly smaller than tropical almond, but the former did not significantly differ with guava. Similarly, mean wing size and shape among the three zones differed significantly (P = 0.000). *B. invadens* from the WMHF had relatively bigger wings, while the NMF recorded the smallest wings. CVA discriminant functions showed a very high degree of zonal population classification (95.48%) accuracy. LVC population wing shape was significantly different from NMF (P = 0.000) and WMHF (P = 0.000), while the latter two were also different (P = 0.000). There were significant differences in allometry and developmental instability fluctuating asymmetry among fruit hosts in the order: mango > guava > almonds. The results have added to the evidence that geographic and host-associated adaptation can produce reproductive isolation and produce ecotypes. The ability of *B. invadens* to undergo rapid ecological evolutionary radiations might affect the efficiency of common control methods, and our results should help to recast fruit fly management strategies.

KEYWORDS: *Bactrocera invadens*, ecotypes, fluctuating asymmetry, hosts, intra-specific variations, tephritid fruit flies

INTRODUCTION

Bactrocera invadens (Drew, Tsuruta & White, 2005) is the most important polyphagous tephritid pest in the region (Mwatawala *et al.,* 2006; Rwomushana *et al*., 2008; Isabirye *et al.,* Unpublished data). This invasive species, probably of Asian origin, is spreading very fast throughout the African continent. The pest (*B. invadens*) belongs to the *B. dorsalis* species complex, which harbors a number of important pest species, some of which are regarded as the most destructive horticultural pests (Drew & Hancock, 1994; Clarke *et al.,* 2005; Schutze *et al.,* 2011). The high polyphagous and invasive nature of *B. invadens* calls for urgent intervention. Nonetheless, because of the cryptic nature of most species in Bactrocera complex, identification of some species is difficult and is of ongoing concern (Clarke *et al.,* 2005). Pest misidentification can have drastic and costly consequences for pest management (Menken & Ulenberg, 1987; Walter, 2003; Drew *et al.,* 2008; Billah *et al.,* 2008; Schutze *et al.,* 2011). Further, it is not clear whether allopatric and host associated speciation has taken course among *B. invadens* populations in the different zones in Uganda, yet this would be important in the design of efficient management strategies. Among the mango growing zones, several factors important for ecological nichie of fruit flies vary and could influence *B. invadens* allopatric and host associated speciation.

Intra-specific variations that lead to the emergence of biotypes, hosts or pheromone races may cause different reactions to control methods (Menken & Raijman, 1996). Earlier on, Mayr & Aschlock (1991) and recently, Kirkpatrick & Ravigne (2002) proposed that speciation allopatric & sympatric have a role during the divergence of populations. In allopatric speciation, geographic barriers among distinct populations may prevent gene flow (Mayr & Aschlock, 1991), resulting into divergence in ethological (Mitani *et al*., 1999), physiological (Tauber *et al*., 1986; Ruberson *et al*., 2001) and morphological (Dujardin *et al.,* 1999; Rodrigues & Moreira, 2002; Jennions & Kelly 2001) characters in any isolated population. In sympatric speciation variation may be the result of adaptation to different habitats or hosts in some localities; which in the presence of assortative mating might decrease gene flow among such populations, resulting into species ecotypes (Kirkpatrick & Ravigne, 2002).

Recent studies on the phylogeny and phylogeography of generalist polyphagous species have hypothesized that most of these species represent complexes of genetically differentiated host races or cryptic species (Nason *et al.,* 2002). If host species constitute different selective regimes to herbivorous insects, genetic differentiation and host-associated local adaptation may occur (Ruiz-Montoya *et al.,* 2003), which is definitely important in pest management programs. Tephritid fruit flies (Diptera: Tephritidae) are highly diverse with over 4, 200 species distributed worldwide (White & Elson-Harris, 1992), and cause tremendous economic damage. Among these, the tropical fruit flies (Tephritidae: Dacinae) are believed to have speciated prolifically throughout the tropics and subtropics (Clarke et al., 2005), with groups of sibling species identified (Drew, 1989; Drew & Hancock, 1994). Host fidelity and consequent sympatric speciation have also been reported in the genus Rhagoletis (*Diptera: Tephritidae)* (Feder *et al.,* 1993). Nevertheless, many of these sibling complexes remain difficult to identify (Schutze *et al.,* 2011). Polyphagous tephritid siblings can potentially vary in important traits, such as host use and pest status, geographic distribution and seasonal phenology. Unfortunately, their study and management remains confounded due to high levels of morphological similarity (Clarke *et al.,* 2001; Schutze *et al.,* 2011). Importantly, mating behavior will also vary among sibling species, which has implications for the sterile insect management technique for which the mating success of treated insects is critical (Dyck *et al.,* 2005).

Identification difficulties chiefly arise due to broad intraspecific morphological variation between members of the *B. dorsalis* complex, which can seriously confound their identification (Drew *et al.,* 2008). Circumventing such challenges requires pursuance of new methods and tools with high resolution and discrimination abilities suited for detecting fine-scale differences which may be expected following recent radiations (Shaffer & Thomson, 2007). One such approach is shape analysis, the quantification of the relative positions of homologous structures, e.g. landmarks or outlines, and then comparing variation in shape data between individuals or groups, a technique known as geometric morphometrics (Rohlf & Marcus, 1993; Rohlf, 1999). This technique has been applied across a range of taxonomic levels, from revealing cryptic species to discriminating intraspecific populations and to resolving relationships at the generic level (Gilchrist & Crisafulli, 2006; Bouyer *et al.,* 2007; Marsteller *et al.,* 2009; Michez *et al.,* 2009). In particular, geometric morphometric techniques focus directly on shape variation and provide potentially greater taxonomic utility over traditional techniques, as shape is regarded as a more heritable character than size (Dujardin *et al.,* 2003).

In addition, continuous characters, such as shape, are hypothesized to be among the first to show differences following isolation events (Bouyer *et al.,* 2007). Therefore, sensitivity to small shape changes between populations may be particularly useful for detecting differences between groups of organisms following recent evolutionary radiations (Schutze *et al.,* 2011). Given the recent invasion of *B. invadens* in Africa (Drew, Tsuruta & White, 2005) it is possible that it has undergone a recent radiation as is the case among some *B. dorsalis* species (Clarke et al., 2005). Geometric morphometric analysis, therefore, may be sensitive enough to resolve differences should such recent radiation exist. Therefore, the objective of this study was to use geometric morphometric techniques to determine the differences and/or similarity among three geographic and hosts *Terminalia catappa, Psidium guanjava* and *Mangifera indica* populations and assess allometry of *B. invadens*. We also examined fluctuating asymmetry FA, small random departures from perfect symmetry among individuals to determine whether individuals reared from different hosts and geographical regions would show significant variability in FA. Ultimately, results of this study will largely help in design and implementation of a knowledge based and sustainable IPM strategies for the three agro ecologies, and the country at large.

MATERIALS AND METHODS

Study area: The study was conducted in three agro ecological zones: Western Medium High Altitude Farmlands WMHF, Lake Victoria Crescent LVC and Northern Moist Farmlands NMF (Wortman & Eledu, 1999). The three zones represent the major fruit growing regions in Uganda (Figure 1). LVC encompasses areas along Lake Victoria that range in altitude from 1100 to 1400 meters above sea-level masl. Rainfall in the area is evenly distributed and ranges between 700-2100mm annually. Banana and coffee are main crops that are often intercropped with annual and biennial root crops sweet potato, cassava and vegetables and fruits. Crops are commonly grown as polycultures on plots less than one hectare. The NMF stretches from Kumi to Kitgum districts, in northern Uganda. Altitude range is small (1000 -1200 masl). The area is dominated by annual crops, mainly cotton, cereals, and cassava. The area records relatively higher temperatures Min. 15^{o}c and relatively uncertain rainfall (700-1700mm/ annum); most species hosts recorded in other zones are less frequent in the zone. Where orchards and areas of substantial fruit growing is done, the areas are not surrounded by reasonable fruit habitats that would serve as important refuges and source areas for alternative hosts for fruit flies during fruit off season. The WMHF zone is located in western Uganda, along the Congo border has an average altitude of 1,235 masl with a range of 600-4500 masl, and receives rainfall ranges of 1000-1200 mm per annum. Mean temperature and rainfall vary across zone, with rainy seasons in March-May and August–November. The main crops grown are bananas, coffee, beans, maize and a variety of vegetables and root crops. Bananas, coffee and cotton are farmers' main source of income.

Sampling Procedure: In each agro ecological zone, representative orchards were selected for lure trapping. In each AEZ, three orchards or areas with substantial mango growing and other potential hosts were selected to represent the major sampling sites. An insecticide strip of dichlorovos was placed in each trap to kill the adult flies. The traps were emptied on a fortnight basis and the lures renewed bi-monthly. Fruit flies were collected and placed into vials containing alcohol for preservation, labelled and transported to the National Agricultural Research Laboratories for identification using multi-entry keys. During the same period, fruits of three types: *Terminalia catappa* (*Combretaceae*), P*sidium guanjava* (*Myrtaceae*) and *Mangifera indica.*

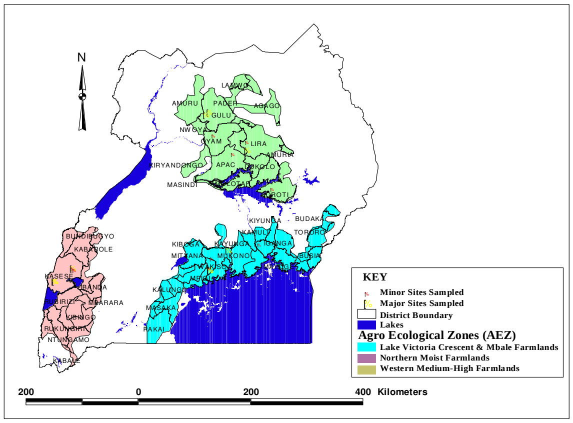

Figure 1: Location of the three Agro ecological zones and the sampling sites in Uganda. The three agro ecological zones are: Western Medium High Altitude Farmlands WMHF, Lake Victoria Crescent LVC and Northern Moist Farmlands NMF Wortman and Eledu, 1993. .

The protocol for collecting, transporting and rearing, of fruit flies followed the methodology developed by the African Fruit fly Programme at ICIPE (Lux *et al.*, 2005), while sample labeling was done according to the protocol described by Copeland *et al.* (2002). Collected fruits were transported to the rearing unit at the National Agricultural Research Laboratories NARL, where they were kept in individual rearing buckets and provided with appropriate medium for pupation as recommended by Copeland *et al.* (2002). The tenneral adults that emerged were carefully removed and handled following methods described by White & Elson-Harris (1992). Confirmation of the identification of the *B. inva*dens that were trapped or emerged from fruits was done at Royal Museum of Central Africa under the guidance of Dr. De Meyer. Voucher specimens are kept in collections at the National Agricultural Research Laboratories, Uganda and Makerere University Zoology Museum and the Royal Museum for Central Africa, Belgium.

Specimen choice, slide preparation and measurement: Only males were examined for all populations, as most available specimens had been collected using the male specific attractant, methyl eugenol. Where females were available (reared fruits), we chose to ignore them to avoid potentially sex biased results (Schutze *et al.,* 2011). Within the limits of the specimens available, samples were chosen to maximize the geographic distance between collection sites so as to increase the amount of potential intraspecific variation measured. All specimens were assigned a cross-referenced code number, which was affixed to both the slide-mounted wing and the pinned or alcohol-preserved voucher specimen. Slide preparation procedure followed guidelines provided by Billah *et al.,* (2008) and Schutze *et al.,* (2011). Thirty specimens each (unless otherwise stated) from the three geographical and host populations each, were dissected using a Leica MS5 Microscope and both left and right wings mounted in Canada balsam as recommended by Shutze *et al.,* (2011). In some instances (guava and tropical almonds), one of the wings was either damaged or missing and so only one wing was mounted. The two wings were taken to assess the possibility of fluctuating asymmetry (differences between the left and right wing) among the geographic and host populations, and to avoid the potential bias of using the left or right wing alone.

Imaging of mounted specimens was done at 10 × magnification using a Sony camera (Model No. DSC-H10, Sony Corp., Japan) mounted onto the Leica MS5 Microscope and saved in JPEG format. Fifteen type 1 landmarks on the wing were selected to represent unambiguous homologous locations on all specimens (Bookstein *et al.,* 1991), and 105 distances between the landmarks computed to characterize the wing as estimations of the size and shape differentiation in the specimens (Figure 2). The selected landmarks followed vein junctions, vein terminations or vein suture: 1 basal junction of veins of cell bm; 2 anterior-most point of the suture located towards the base of vein sc; 3 inner antero-distal corner of cell bc; 4 junction of veins A_1 and CuA_2; 5 junction of CuA_1 and CuA_2; 6 junction of vein CuA1 and dm-bm cross vein; 7 junction of vein M and dm-bm cross-vein; 8 junction of vein CuA_1 and dm-cu; 9 junction of vein M and dm-cu; 10 junction of vein M and r-m cross-vein; 11 junction of vein R_{4+5} and r-m cross-vein; 12 junction of vein R1 and costal vein; 13 termination of vein M; 14 termination of vein R_{4+5}; and 15 termination of vein R_{2+3} (Schutze *et al.,* 2011). Measurements and land mark digitization were done using tpsDig, Version 2.16 software programme (Rohlf, 2010), for which landmark x, y coordinates were generated and saved as a text file.

Data analysis: Raw landmark co-ordinate data were imported into PAST and MORPHOJ v 1.02E programs and aligned using a generalized Procrustes analysis procedure, which is a process for removing non-shape variation i.e. rotation, translation and scale from the data (Rohlf, 1999). Centroid size was used as the measure of size for each fly wing (Hammer et al., 2002). Tests for significant differences among samples for each geographic or host population were undertaken using a one-way ANOVA with a Tukey post-hoc test. As wing size can also significantly influence the shape of the wing allometry, we undertook a multivariate regression of the dependant variable wing shape on centroid size independent variable using the software package MORPHOJ v 1.02E (Klingenberg, 2011; Schutze et al., 2011). The statistical significance of the regression was tested by permutation tests 10,000 replicates against the null hypothesis of independence. Procrustes transformed coordinate data was then used for subsequent Multivariate Analysis of Variance (MANOVA) and canonical variates analysis (CVA), and also for group assignment tests using confusion matrices. The latter test reassigns individuals to an a priori defined group following CVA based on Mahalanobis distances between the individual and group centroids. For CVA, individuals were a priori defined as members of one of the 3 geographic or host populations based on collection locality or fruits from which it was reared. Following CVA, intraspecific groups were combined according to populations to form homogenous groups, for which the assignment test was undertaken to determine the number of individuals from any one sample being reassigned to their a priori population. Three Fluctuating asymmetry (FA) indices FA3, FA7 & FA9 recommended by Palmer & Strobeck (1986) were computed. Fluctuating asymmetry was calculated as the absolute value of the difference between the left (L) and right (R) sides for the wing size trait. FA3 is the mean of the absolute value of the unsigned difference between left and right ($FA = |L - R|$), divided by the population trait size mean, FA7 is the variance of the signed difference, divided by the population trait size mean, while FA9 contains the square of correlation coefficient $1-r^2$.

RESULTS

Effect of host type on intraspecific wing size and shape variation: One way-Analysis of variance ANOVA for the effect of host type on wing size showed significant difference among the three hosts ($F = 21.31$, $df = 2, 159$, $P = 0.0001$). Mango wings were significantly smaller than tropical almond, but the former did not significantly differ with guava (Figure 2). Wing size relative variability among the three hosts followed a reverse order to size: Mango > Guava > Almonds (Figure 2). Multivariate analysis of variance MANOVA of Euclidean distance EDMA between all pairs of the 15 landmarks indicated significant differences among the three hosts (Wilks' λ=0.07186, df = 202, 118, P = 0.003). Subsequent Canonical Variates Analysis CVA of specimens along the two first canonical axes, producing maximal and second to maximal separation between all groups showed appreciable host population discrimination (Figure 3). The first two canonical variates contributed a total of 97.8% (CV 1 = 70.5% and CV 2 = 27.3%) to the total variance. CVA discriminant functions showed a very high (95.03%) degree of host population classification accuracy. The Bray-Curtis dissimilarity measure showed overall population dissimilarity of 1.678, with subsequent pair wise differences following the order: Guava vs. Mango > Mango Vs Almonds > Guava > Almonds. Mean Landmark distances of the ten distances contributing most to the differences among the three hosts are shown in Table 1 below. Distances between landmarks 2-4, 1-4 and 3-4 contribute highest to the differences. Procrustes ANOVA of the effect of host type on wing shape showed significant wing shape differences among the three hosts (F = 21.31, df = 52, 4134, P = 0.0001). The first two principal axes showed partial or fuzzy separation of the populations Figure 4. Subsequent projection onto the first two canonical axes distinctly separated the populations into the three hosts clusters (Figure 5). The first two canonical variates contributed a total of 100.0% (CV 1 = 72.5% and CV 2 = 27.5%) to the total variance (Figure 5). Post-hoc analysis showed that Almonds wing shape was significantly different from Guava (Mahalonobis distance = 2.0680, P = 0.000) and Mango (Mahalonobis distance = 1.4315, P = 0.000), while the latter two were also different (Mahalonobis distance = 1.6063, P = 0.000). The trend for the difference under procrustes distance was the same. Discriminant function analysis with mahalanobis and procrustes distances confirmed the significant differences between the three host populations (P = 0.000). Success rate for the reassignment of individuals from any one sample to their a priori defined species ranged from 74.55% (Mango) to 86.61% (Guava), with Almonds being intermediate to the latter two (79.41%). Multiple regression revealed appreciable allometry as the relationship between wing shape and centroid size was significant (P = 0.0052). Post-regression analysis of the means of the three host's shape-centroid size relationships showed that Almonds and Guava did not differ significantly in shape, but the two were significantly different from Mango shapes (Figure 6). Indeed of the three host's wing shapes, wing size significantly affected most the Mango based *B. invadens* than the other two hosts, as indicated by the highest means confidence ellipse (Figure 6).

Table 1 Mean Landmark distances of the ten distances contributing most to the differences among the three hosts

Distance	Contribution	Cumulative %	Guava	Mango	Almonds
2_4	0.03443	10.2	0.275	0.274	0.267
1_4	0.03406	10.0	0.248	0.249	0.242
3_4	0.03253	9.6	0.300	0.298	0.292
4_8	0.03114	9.2	0.291	0.292	0.297
4_5	0.02929	8.6	0.138	0.137	0.131
4_7	0.02857	8.4	0.189	0.187	0.180
4_13	0.02813	8.3	0.540	0.539	0.545
12_15	0.02513	7.4	0.196	0.204	0.194
4_9	0.02446	7.2	0.361	0.360	0.361
4_6	0.02442	7.2	0.121	0.120	0.115
11_15	0.02366	7.0	0.235	0.245	0.246
14-15	0.02322	6.8	0.189	0.182	0.183

Intraspecific geographical variations in wing size and shape: Mean wing size among the three zones differed significantly (F = 8152.27, df = 2, 152, P = 0.000). *B. invadens* from the WMHF had relatively bigger wings, while the NMF recorded the smallest wing sizes (Figure 7). Similarly, wing size relative variability among the three zones followed a reverse order to size: NMF > LVC > WMHF Figure 2. MANOVA of all the EDMA distances between all pairs of the 15 landmarks indicated significant wing differences among the three zones (Wilks' λ=0.0517, df = 202, 96, P = 0.008). Subsequent projection of the three populations along the two first canonical axes, depicted significant ecotype clusters Figure 8. CVA discriminant functions showed a very high degree of host population classification (95.48%) accuracy. The first two canonical variates contributed a total of 100% (CV 1 = 58.91% and CV 2 = 41.09%) to the total variance. The Bray-Curtis dissimilarity measure showed overall population dissimilarity of 1.582, with subsequent pair wise differences following the order: NMF vs. LVC > WMHF Vs LVC > WMHF > NMF. Mean Landmark distances between landmarks 12-13, 1-12 and 7-12 contributed highest to the differences in zonal populations (Table 2).

Table 2 Mean Landmark distances of the 20 wing distances contributing most to the differences among the three populations.

Taxon	Contribution	Cumulative %	WMHF	NMF	LVC
12_13	0.02924	6.6	0.181	0.181	0.193
1_12	0.02631	5.9	0.188	0.193	0.186
7_12	0.02611	5.9	0.238	0.238	0.240
10_13	0.02495	5.6	0.539	0.541	0.546
3_4	0.02372	5.3	0.562	0.562	0.566
5_12	0.02299	5.2	0.597	0.598	0.603
2_4	0.02262	5.1	0.361	0.365	0.371
1_4	0.02251	5.1	0.244	0.243	0.239
11_13	0.02242	5.0	0.360	0.362	0.354
4_13	0.02159	4.9	0.449	0.448	0.452
6_12	0.02147	4.8	0.237	0.239	0.239
11_12	0.02134	4.8	0.384	0.384	0.376
4_12	0.02090	4.7	0.497	0.496	0.500
11_14	0.02081	4.7	0.455	0.455	0.458
3_11	0.02037	4.6	0.478	0.478	0.482
4_5	0.02022	4.6	0.383	0.381	0.386
4_7	0.01995	4.5	0.425	0.426	0.418
9_13	0.01905	4.3	0.28	0.278	0.271
3_10	0.01876	4.2	0.344	0.347	0.341
7_10	0.01873	4.2	0.315	0.311	0.312

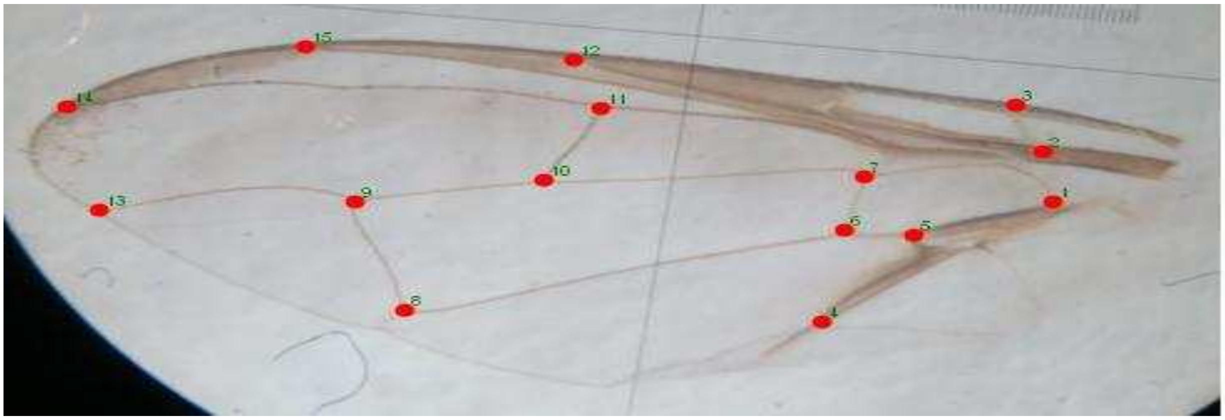

Fig 2 Left-hand wing of a *Bactrocera invadens* individual showing each of the 15 landmarks used in the geometric morphometric analysis. 1, basal junction of veins of cell bm; 2, anterior-most point of the suture located towards the base of vein sc; 3, inner antero-distal corner of cell bc; 4, junction of veins A1 and CuA_2; 5, junction of CuA_1 and CuA_2; 6, junction of vein CuA_1 and dm-bm cross vein; 7, junction of vein M and dm-bm cross-vein; 8, junction of vein CuA_1 and dm-cu; 9, junction of vein M and dm-cu; 10, junction of vein M and r-m cross-vein; 11, junction of vein R_{4+5} and r-m cross-vein; 12, junction of vein R_1 and costal vein; 13, termination of vein M; 14, termination of vein R_{4+5}; 15, termination of vein R_{2+3}

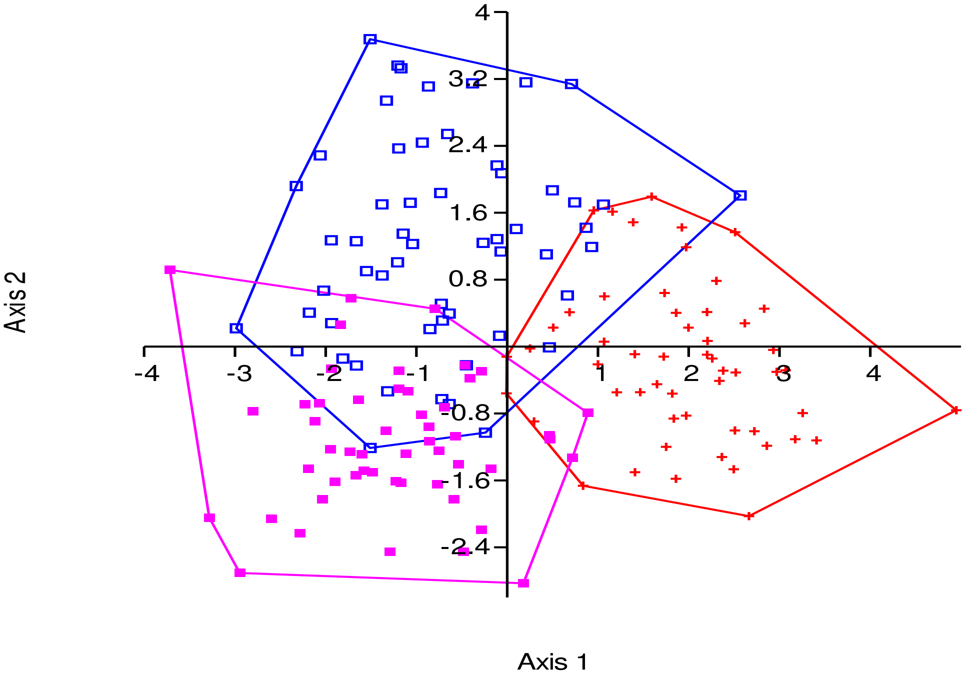

Fig 3 Canonical Variates Analysis of the effect of three hosts on wing centroid size for the three populations showing the three clusters.. (Blue squares: Mango; Pink squares: Tropical almonds; Red crosses: Guava). The ellipses show convex hulls for each population.

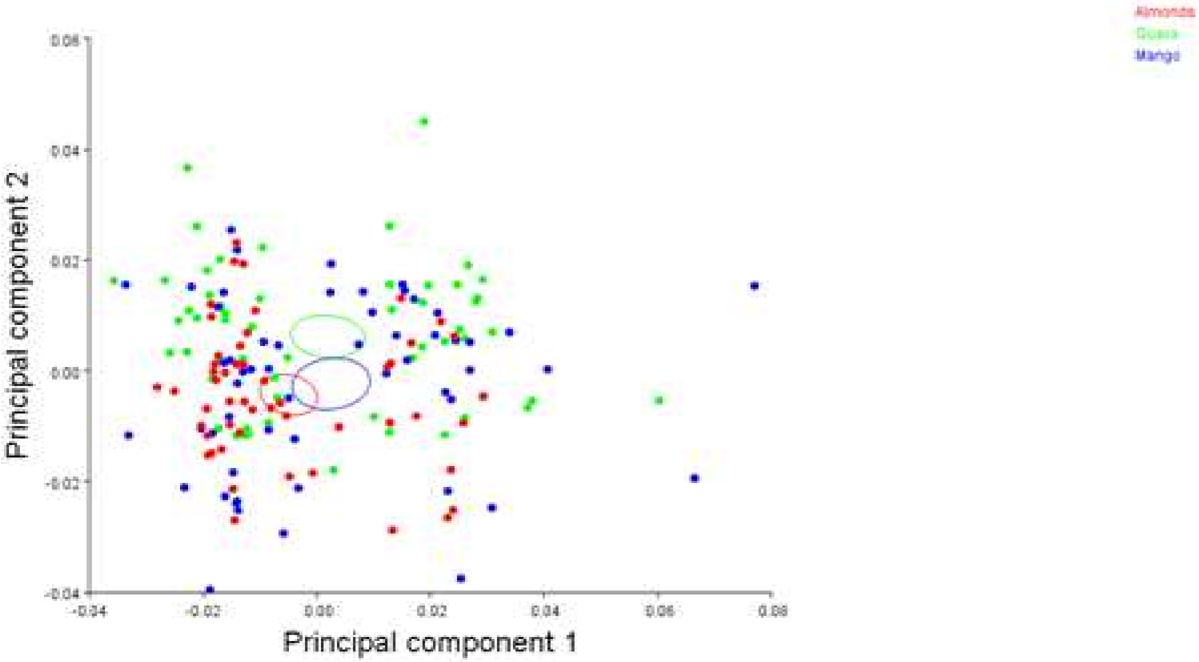

Fig.4 Projection of wing data of the three *B. invadens* populations on the first two principal components showing a fuzzy separation of the populations. Blue points: Mango; Pink points: Tropical almonds; Red points: Guava. The ellipses show 90% means confidence intervals for each population.

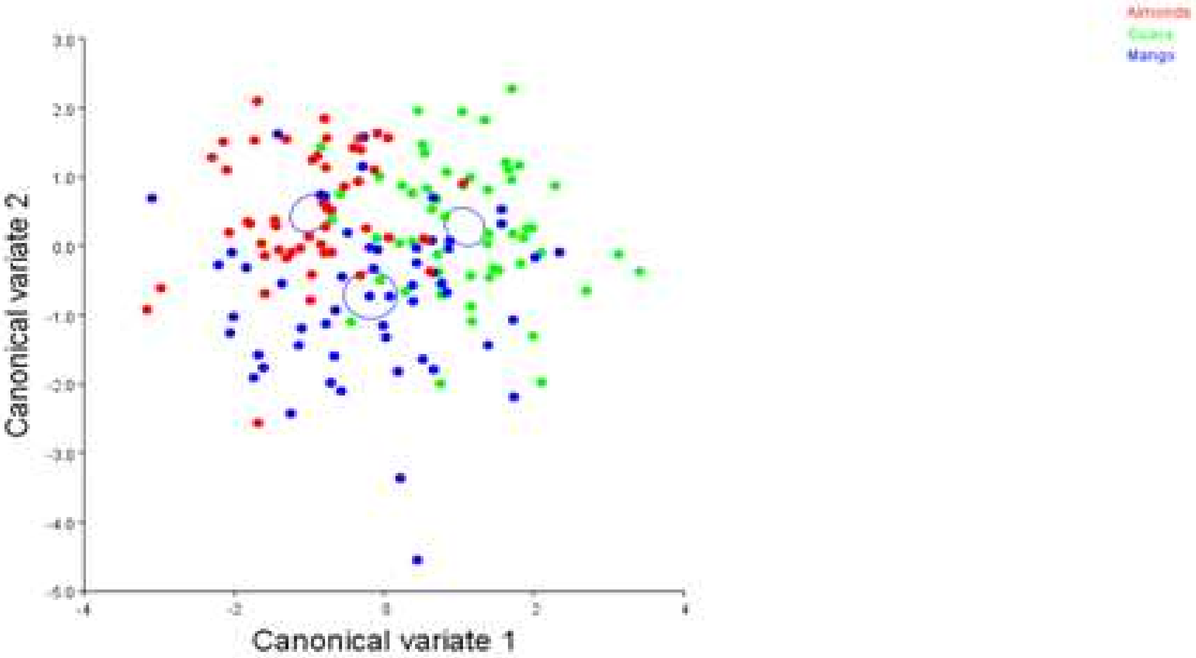

Fig 5. Canonical Variates Analyisis of the effect of the three hosts on wing centroid size showing the three clusters. (Blue points: Mango; Pink points: Tropical almonds; Red points: Guava). The ellipses show 90% means confidence intervals for each population.

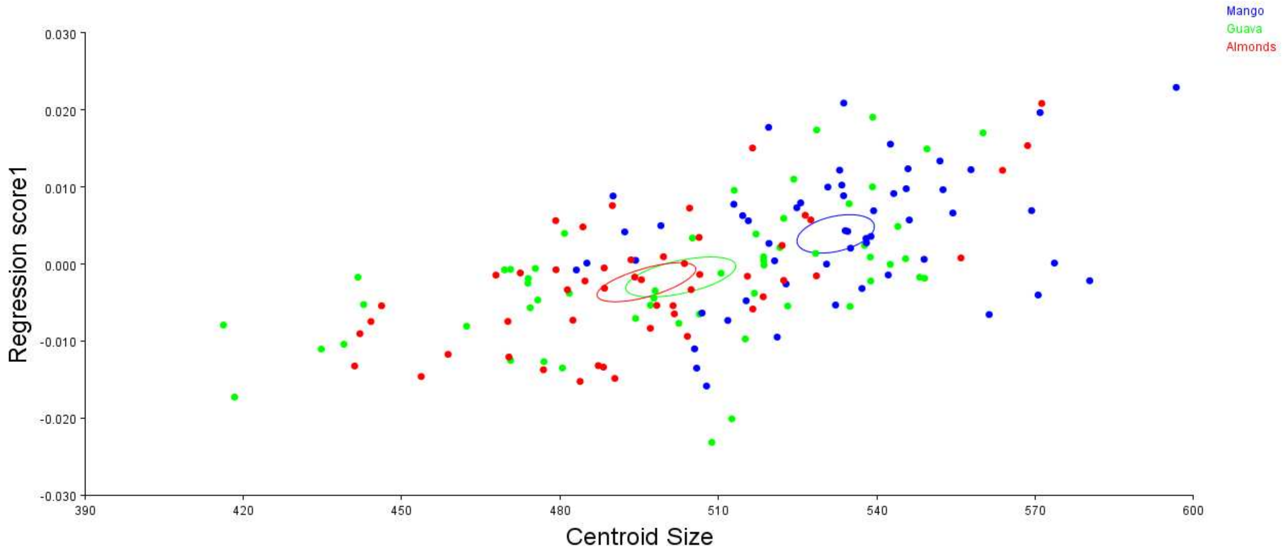

Fig 6. Multivariate regression of wing shape Regression scores 1 on centroid size wing size. Blue points: Mango; Pink points: Tropical almonds; Red points: Guava. The ellipses show 90% means confidence intervals for each population.

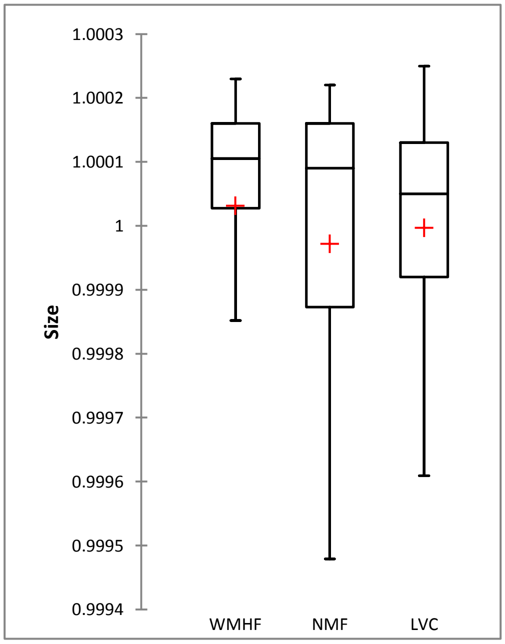

Fig. 7 Effect of zones on fruit fly wing size (centroid). Box plots show median, mean, 25th and 75th percentiles, and 10th and 90th percentiles.

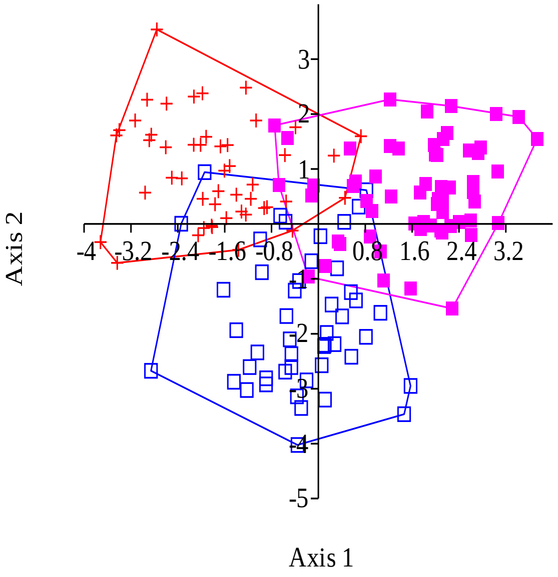

Fig. 8 Canonical Variates Analyisis of the effect of three zones on wing centroid size showing the three clusters.. (Blue squares: Mango; Pink squares: Tropical almonds; Red crosses: Guava). The ellipses show convex hulls for each population.

Geographical location significantly affected wing shape too across the three zones (F = 2.77, df = 52, 3952, P = 0.000). The first two principal component axes with fuzzy separation of the populations are shown in Figure 9. The first two canonical axes distinctly separated the populations into their respective ecotypes, with the first two canonical variates contributing a total of 100.0% (CV 1 = 53.608% and CV 2 = 46.392%) to the total variance (Figure 10). Post-hoc analysis showed that LVC population wing shapes were significantly different from NMF (Mahalonobis distance = 1.6673, P = 0.000) and WMHF (Mahalonobis distance = 1.5955, P = 0.000), while the latter two were also different (Mahalonobis distance = 1.6985, P = 0.000). Discriminant function analysis with mahalanobis and procrustes distances confirmed the significant differences between the three geographic populations (P = 0.000). Wing shapes discriminant function reclassification success rate for the three populations ranged from 81.67% (LVC) to 84.78% (WMHF), with NMF being intermediate to the latter two (83.68%). Multiple regression revealed significant allometry across the three populations (P = 0.046). Post-regression analysis of zonal means shape-centroid size relationships showed that LVC and NMF did not differ significantly in allometry, but the two were significantly different from WMHF (Figure 11).

Fluctuating Asymmetry FA in *B. invadens* allopatric and host populations: There were significantly higher levels of FA in mango host, compared to almonds and guava populations, but post-hoc analysis did not show significant difference between the latter two with FA3 and FA7 indices (Table 3). With the exception of left wing among the three zones, both right and left wings among the three hosts and the right wings in the zonal specimens showed inverse significant relationships between absolute fluctuating asymmetry and wing size (Zones: r = -0.774, P = 0.000, R^2 = 59.9%); hosts right: r = -0.609, P = 0.000, R^2 = 37.1%), and left: r = -0.551, P = 0.000, R^2 = 30.4%). The relationship was strongest in the NMF (r = -0.825) and least in WMHF (r = -0.741). At the host level, the relationship was significant for both wings among guava reared flies, while for mango and almonds it was only in left and right wings, respectively. All fruit host populations depicted a bilateral fluctuating asymmetry type (Table 3; Figure 12). No significant differences in FA among the three allopatric populations were detected, although there were variations in levels of bilateral FA in the order: NMF > LVC > WMHF (Table 3). All zonal populations pooled showed a fluctuating asymmetry, but there were differences in forms of bilateral asymmetries. The LVC followed bilateral fluctuating asymmetry type; NMFs depicted a skewed antisymmetry form, while the WMHF followed a directional asymmetry form (Figure 12).

Table 3 Comparison of fluctuating asymmetry FA among fruit hosts and zones. Subscript R ± L denotes difference in value measured on left and right side, TS denotes trait size = average of values of left and right side Palmer & Strobeck, 1986.

	N	FA3 $\mu\lvert R-L\rvert / \mu TS$	FA7 $\sigma^2 R\text{-}L/\mu TS$	FA9 $1\text{-}r^2/\mu TS$	Asymmetry FA type
Hosts					
Guava	28	1.845E-04 ab	9.447E-08 ab	0.98814a	Fluctuating
Mango	25	2.695E-04 b	1.767E-07 b	0.97451a	Fluctuating
Almonds	23	1.168E-04 a	2.939E-08 a	0.98913a	Fluctuating
Test/ Prob.		F=2.662, P = 0.042	X^2 = 15.838, P = 0.000		
All Fruits	76	1.903E-04	1.031E-07	0.98748	Fluctuating
AEZs					
WMHF	15	1.895E-04 a	1.078E-07 a	0.99998a	Directional
NMF	18	2.056E-04 a	7.863E-08 a	0.80638a	Antisymmetry
LVC	29	1.928E-04 a	8.934E-08 a	0.99188a	Fluctuating
Test/ Prob.		F = 0.021, P = 0.979	X^2 = 9.991, P = 0.808		
All Zones	62	1.957E-04	9.10749E-08	0.97422	Fluctuating

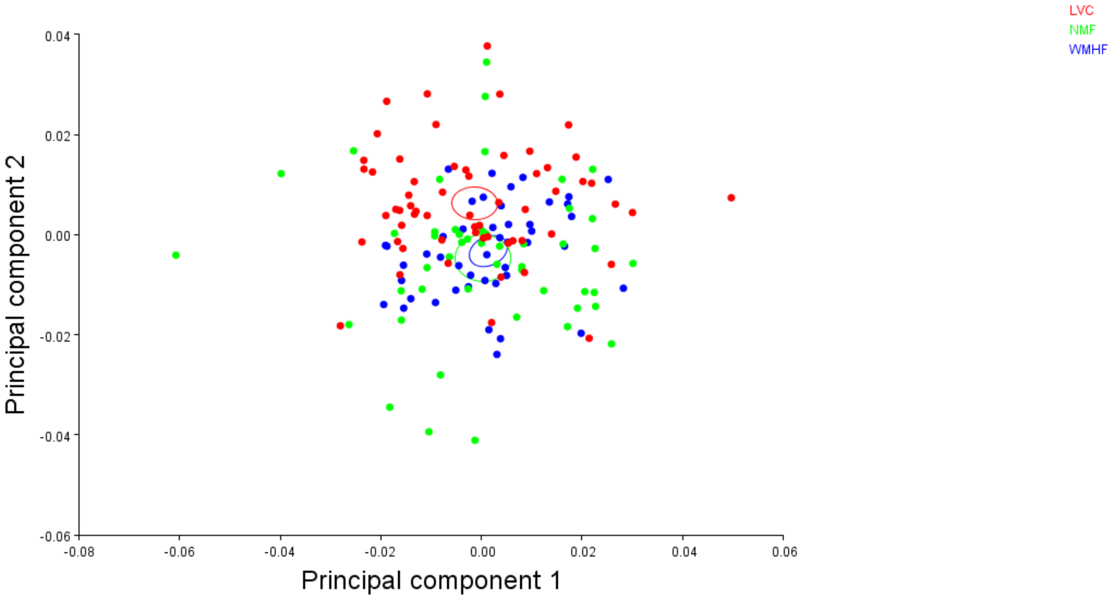

Fig 9. Projection of wing data of the three *B. invadens* zonal populations on the first two principal components showing a fuzzy separation of the populations. Blue points: WMHF; Red points: LVC; Green points: NMF. The ellipses show 90% means confidence intervals for each population.

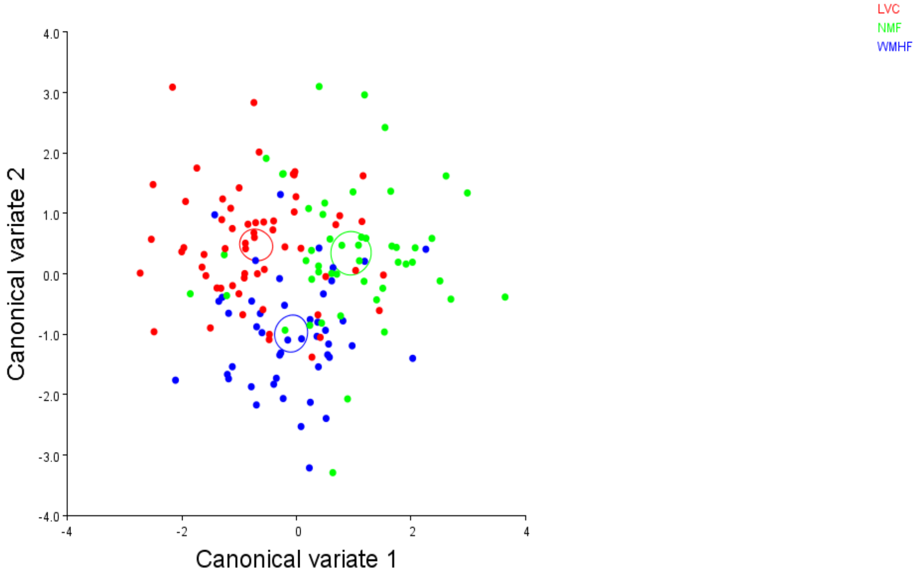

Figure 10. Canonical Variates Analyisis of wing shapes for the three populations. (Blue points: WMHF; Red points: LVC; Green points: NMF). The ellipses show 90% means confidence intervals for each population.

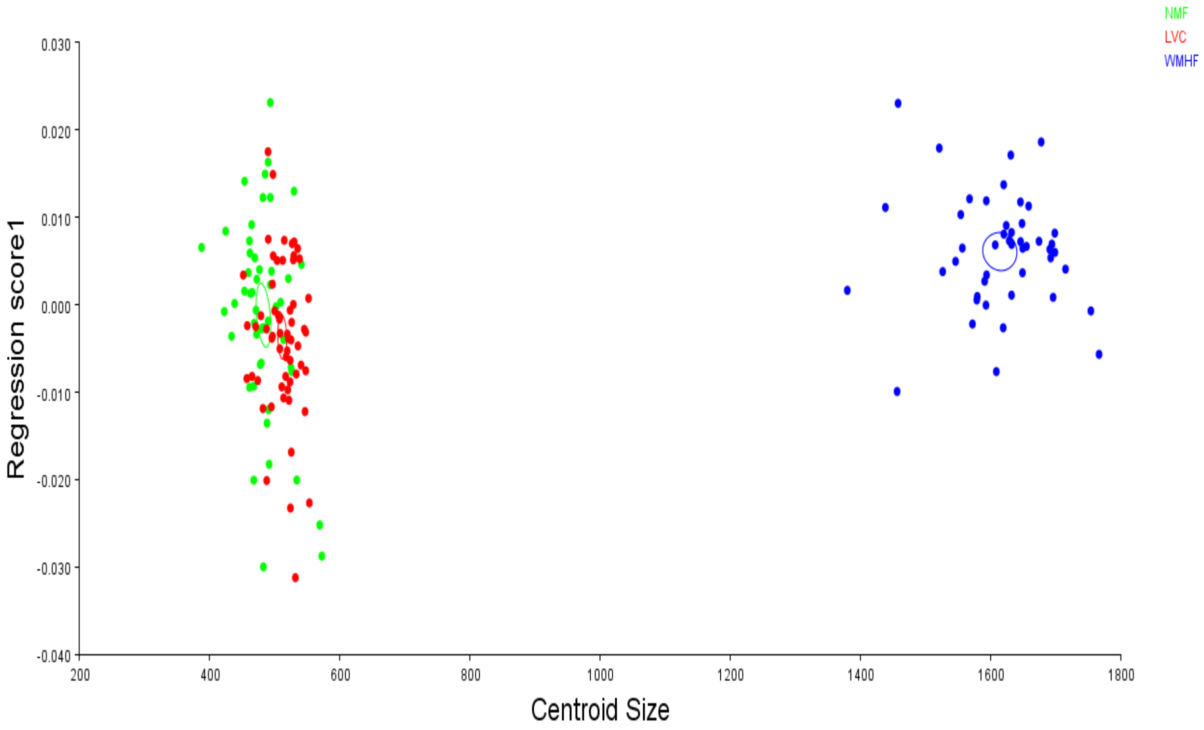

Fig 11. Multivariate regression of wing shape Regression score 1 on centroid size wing size of three populations of *B. invadens*. Blue points: WMHF; Red points: LVC; Green points: NMF. The ellipses show 90% means confidence intervals for each population

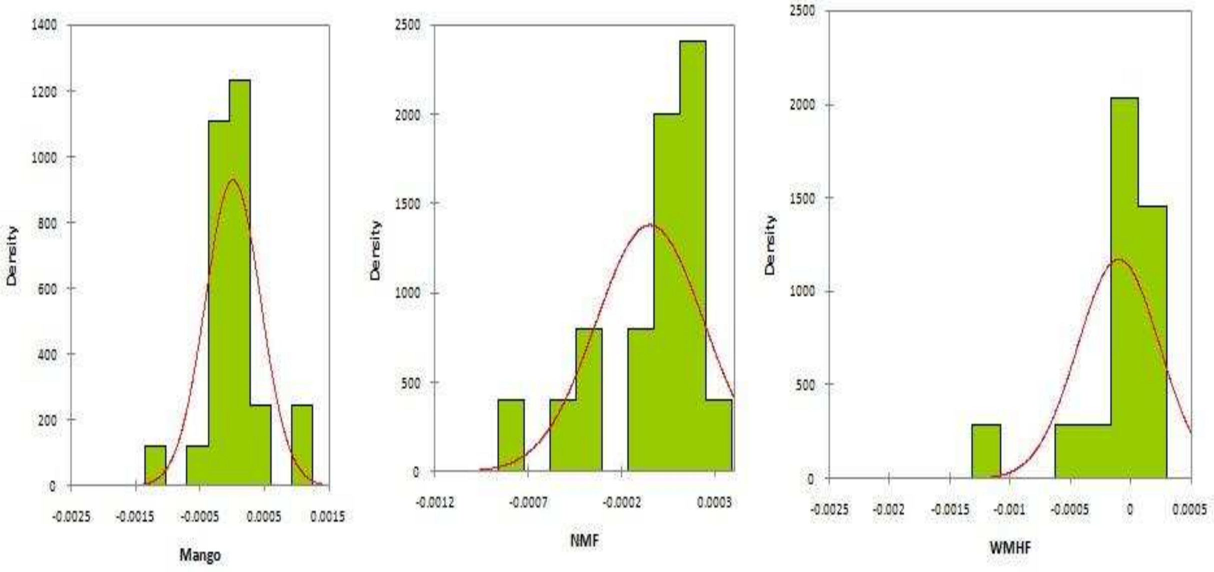

Fig 12. Histograms depicting the three forms of bilateral asymmetry: Fluctuating asymmetry Mango, Antisymmetry NMF and Directional asymmetry WMHF.

DISCUSSION

Intraspecific wing size and shape variation: The aim of this study was to determine the differences and/or similarity among three geographic and hosts, using Euclidean distance matrix analysis and variance structure coordinate system geometric morphometrics techniques. The results generally significant levels of intraspecific population variations and added to the evidence that geographic and host-associated adaptation can produce reproductive isolation and produce ecotypes. The application of geometric morphometric (GM) techniques in insect species comparison studies has been widely used and supported as a promising approach for discriminating between morphologically cryptic species and complexes (Daly, 1985; Woolley & Browning, 1987; Woolley *et al.,* 1994; Umphrey, 1996; Wool & Hales, 1997; Adsavakulchai,1998; Dujardin *et al.,* 2003; Gilchrist & Crisafulli, 2006; Drew *et al.,* 2008; Billah *et al.,* 2008; Kitthawee & Dujardin, 2010; Schutze *et al.,* 2012). As in most of the preceding studies, we also found GM a very convenient method for separation of closely related species of insect. Particularly, GM is advantageous as it can work with both fresh and museum preserved specimens, requires relatively less taxonomy skills, output data formats can be utized in multiple forms, and the techniques opportunities for on-line biological classification scheme which may have implications in the field of agricultural entomology particularly in the tropical regions (Adsavakulchai,1998).

The high intraspecific morphological variations in our study is not surprising, as many previous studies have reported similar results among *B. dorsalis* complex members, of which *B. invadens* is part (Drew *et al.,* 2008; Adsavakulchai,1998; Schutze *et al.,* 2012). Dacinae fruit flies are projected to have originated in the Papua New Guinea area and speciated prolifically across major world regions (Drew, 1989). This rapid species has led to emergency of cryptic and sibling species, which high levels of similarity in external morphology and geographic/ host variation in morphology within each species (Drew, 1989; Adsavakulchai,1998). Recently, Schutze *et al.,* (2012) reported significantly different intraspecific wing size and shape, with canonical variate analysis discriminating B. dorsalis species with a relatively high degree of accuracy (93.27%). This very comparable to our results: significant intraspecific population discrimination among hosts and ecotypes were recorded, with average reclassification accuracies of 95.03% and 95.48% for host and ecotype populations, respectively.

However, unlike in the studies by Schutze *et al.,* (2012), wing centroid size in our case was found to effectively discriminate populations, both with EDMA and convariance matrix approaches. The three fruit hosts and zones from which the samples were taken present significant variability in environmental and larval food sources (*ibid*). Consequently, it is not surprising to record intraspecific variations, as body size in tephritids is considered to be heavily influenced by host types and phenology, and environmental conditions (Hooper, 1978; Krainacker *et al.,* 1987). Such variation in intraspecific sizes between populations may explain the difference in wing sizes observed among host ang geographical populations.Therefore, where post-hoc differences were not significant (e. g mango vs. guava), it is likely that such hosts expose the larvae to similar environmental conditions, possibly resulting in adults of similar size (Schutze *et al.,* 2012). Indeed specimens from mango and guava have similar EDMA distances between the major landmarks 2-4, 1-4, 3-4, 4-8 compared to almonds (Table 1). The high variation among individuals in our study maybe evidence of different genotypes in the ecotype populations. It seems that there is enough genetic variation in natural populations of *B. invadens* to show alternative phenotypes in response to hosts and geographical variations. The *B. invadens* populations host overlap and the resultant variations observed may lead populations to adapt and survive in difficult conditions such as the stress caused by control practices. A similar study on intra population variation and gene flow among pest populations of by (Sluss & Graham 1979) concluded that populations may rapidly respond to control measures and subsequent resistance would be spread throughout populations, as shown in natural populations.

Although our findings generally show significant population discrimination, caution is needed when deciding to use wing-based shape data. We can not at this stage confirm or refute species limits, but rather present this as one line of evidence along with other species delimiting data such as mating and genetic studies. Therefore, fine-scale intraspecific populationvariations in our study may simply be evidence of divergences occurring during the early stages of evolutionary radiation, at a stage which is concomitant with inter-population, rather than interspecific, differences (Schutze *et al.,* 2012). Such differences may be unresolvable using techniques such as molecular analysis, hence the current lack of molecular markers to discriminate between these species. As already reported elsewhere (McInnis et al., 1999;Wee &Tan, 2005), the high degree of intraspecific variations in our study, combined with pheromone and cross-mating data suggesting hybridization among *B. dorsalis* species, further emphasises the possibility that these taxa represent species in the early stages of divergence rather than distinct and reproductively isolated biological species. Therefore, rather than provide fine answers, our study has set the debate that will require more taxonomic studies in correlation with population genetic and cytotaxonomy investigations of the *B. invadens* to elucidate and resolve the fine identities of cryptic or isomorphic species.

Allopatric and host-associated fluctauating asymmetry: Within populations of animals with an essentially bilaterally symmetrical body plan, minute deviations to the left or right, or the difference in value between paired bilateral traits, are known as fluctuating asymmetry FA (Van Valen, 1962; Markow, 1995). FA is commonly used as a measure of developmental instability DI, which refers to an individual’s inability to produce a specific phenotype under a given set of environmental or other forms of stress conditions (Demontis *et al.,* 2010; McLachlan, 2010). FA is known to be elevated due to environmental stresses and genetic factors and is often seen as an indicator of `overall quality' or `general health' (Zakharov, 1992; Lens *et al.* 2000; Andersen *et al.* 2002; Kristensen *et al.*, 2004; Røgilds *et al.* 2005; Pertoldi et al. 2006; Petavy *et al.* 2006; Soderman *et al.* 2007; Krag *et al.* 2009). The idea behind this concept is that individuals of low quality cannot control their development precisely, and consequently more often develop different phenotypes on both sides (Windig & Nylin, 2000).

FA is characterized by a normal distribution of right-left side differences with a mean of zero (Palmer & Strobeck, 1986). Even though the mean of right and left wing differences were not different from zero, the high level of kurtosis and skewness values among zone and host populations in this study is evidence of FA among *B. invadens* (Cuervo, 2000). Failure to attain perfect 0 mean in our case may be related to the precision of the measurement system used. A lower precision system may only display big differences in R-L measures and overlook the small ones, whereas in very precise systems rarely an individual displays in a trait a R-L of 0 although the global mean is not different of 0 (Cuervo, 2000). However, independent of the measurement technique used, Palmer & Strobeck (1992) indicated that leptokurtic distributions might be obtained as a result of a mix of individuals with low and high FA or a mix of individuals with FA and Antisymmetric Asymmetry (AA), being very difficult to discern both situations, as in our case (Table 3). Related studies have also proved that leptokurtic distributions may be possible and therefore be subjected to environmental stress, as the ideal FA does (Leung & Forbes, 1996). Nonetheless, underestimation of the real level of FA in a population is not a problem when levels of FA are used to compare hosts or ecotypes or to relate them to environmental variables (Demontis *et al.,* 2010; McLachlan, 2010), which gives credensce to our findings.

Although *B. invadens* was able to propage and survive in each of the three hosts, developmental stability inverse of DI was significantly lower higher FA in mangoes than in the other two hosts (Table 3). The reason for the low FA low DI among the almonds population could be attributed to the host inferred buffering mechanism against random noise in the cellular processes among species reared from almonds. Cellular buffering mechanisms are involved in the development of morphological structures (Lynch & Force,2000). DI has been attributed to the inability of an organism to buffer its developmental processes against environmental and genetic disturbances to ensure bilateral symmetry, which would logically imply that mango hosts are relatively poor hosts as they seem not to infer such abilities (Demontis *et al.,* 2010). However, the study recorded an inverse relationship between wing size and FA, which would have implied that *B. invadens* populations reared from mangoes would exhibit less FA, but it was the converse (Figure 2). The mango paradox potentially means that increase in FA in mango might not be due to environmental factors in isolation, but maybe attributed to genetic incompatibilities in mango reared flies (*ibid*). Therefore, the incomplete knowledge of the genetic mechanisms behind FA and host utilization makes clarification of the paradox a challenge, and calls for more efforts to understand how the two processes responsible for intraspecific variation are controlled and interact.

The three indices used in this study support the suggestion that to determine the relationship between asymmetry and environmental stress, a large set of indices should be used, as FA might vary significantly under different variables and indices sets

(Palmer & Strobeck, 1986; Windig & Nylin, 2000; Hogg *et al.,* 2001). The failure to detect the relationship between FA and host types by the FA9 index could be masked by the low sensitivity of the index (Palmer & Strobeck, 1986; Windig & Nylin, 2000) or due to effects of other non-measured factors (Hogg *et al.,* 2001). Consequently, when populations are compared, as was the case in this study, one of the difficulties is to select the characters to be used and to know if results will vary depending on the character (Lajus, 2001). Our choice of centroid size in this study could have masked other development variations, with implications on finding differences in zonal populations (Table 3). We therefore recommend use of multiple traits to refine our findings, but see Hogg et al., (2001). Our findings that indicated relationship between host or site quality support the use of FA as an easy and efficient method to assess the population status under varying stress conditions, and therefore FA could be a useful indicator tool to integrate in the management of fruit flies.

CONCLUSIONS

Once again, GM has revealed capacity to resolve fine-scale intraspecific differences and potential rapid radiation among host and geographical populations at a local scale. GM therefore has reaffirmed its precision and ability to quickly and relatively easily identify morphologically cryptic pest species, and demonstrated its potential for use as a rapid identification tool given a broader, and more comprehensive dataset (Schutze *et al.,* 2012). The results have added to the evidence that geographic and host-associated adaptation can produce reproductive isolation and ecotypes. The ability of *B. invadens* to undergo rapid ecological evolutionary radiations is likely to affect the efficiency of common control methods, and our results should help to recast fruit fly management strategies. The prevalence of fluctauating asymmetry, particularly among hosts, calls for more work to quantify the environmental and host based stress thresholds where population developmental patterns are highly instable, which could aid in the development of management strategies.We recommend further behavioral and ecological research to confirm or refute whether the observed host and geographic differences have genetic and behaivioural confirmatory basis before utilization of these variations in design of efficient diagonistic tools and management strategies.

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