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Shape and Wing Size Symmetry in local populations of *Aedes albopictus* Skuse

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Abstract
A study on wing symmetry in *Aedes albopictus* was done using SAGE (Symmetry and Asymmetry of Geometric Data) software. Fifteen pairs of the left and right wings from each of the sexes of the mosquitoes collected from three locations (coastal, poblacion and rural) of Cagayan de Oro city were mounted on glass slides, microphotographed, digitized and assigned with 20 landmarks with tps Dig software. Thin-plate spline images of the wings were pooled in tps files using tpsUtil and were landmarked and subjected in SAGE software, ver. 1.04 to detect the state of symmetry in *the* wings. Results showed that for shape symmetry, Procrustes ANOVA of data sets for both sexes showed highly significant left-right variations as shown by significant individual, sides, and individual x side's effects. Fluctuating Asymmetry (FA) was detected in both coastal and urban populations in the female but not in the rural population which had significant Directional Asymmetry (DA). For the male populations, DA was detected in the coastal populations but not in the other two populations. The results for the wing size symmetry analysis showed that all populations exhibited FA. These results in *Aedes albopictus* clearly indicate that there was developmental instability in wing size but for the wing shape, the existence of directional asymmetry in two populations – the rural population of the female and coastal population for the male indicate that the variations in symmetry in the mosquito can have a genetic basis.

Keywords: Fluctuating asymmetry, Directional asymmetry, Developmental stability, wing shape, SAGE, Procrustes.

1. Introduction

Bilateral symmetry among animals is rarely perfect, i.e., when you measure left and right body parts, *viz*. wings of flies, their lengths or widths do not always measure perfectly equal. During growth and development, the perfect symmetry of bilateral structures often becomes thrown out of balance in subtle or sometimes obvious ways. Bilateral asymmetry may be observed to occur in three general patterns of left-and-right structural differences in a population, *viz*: fluctuating asymmetry (FA), directional asymmetry (DA) and antisymmetry (AS) [1, 2]. FA is a random deviation from bilateral symmetry, DA involves repeatable deviations from symmetry towards the same side, and antisymmetry is bimodal asymmetry that is random with respect to side. One morphological structure that brought interest to investigate morphological forms is the insect wing. Insect wings are considered reliable indicators for variation studies because they are immutable to short-term micro-environmental effects (e.g. competition and temperature) and yet susceptible to long-term microevolutionary effects (e.g. genetic drift, mutation, and natural selection) [3, 4, 5]. Insect wings are ideal for morphometric studies because the wing structures provide discrete anatomical loci (landmarks) that can be digitized and subjected to vigorous statistical analyses [6, 7]. The phenotype of insects is under genetic and environmental control, thus, the analysis of variations in landmark configurations of the wings of insect populations may provide relevant information about many aspects of insect biology such as the development of wing size and shape. Recent studies have shown interest to the examination of asymmetry in body plan of organisms as an indicator of individual quality, thus, this current study was investigated on the type of matching symmetry in both sexes of *Aedes albopictus* collected from locations which vary geographically and community type (coastal, poblacion, and rural areas) of Cagayan de Oro City to determine the kind of asymmetry in the populations that may be attributed to population-level stress (FA) or may have genetic basis (DA).

2. Methodology

Adult mosquitoes collected in different locations – rural, poblacion and coastal areas in Cagayan de oro city using the Prokopack Aspirator (Model 1419 from John W. Hock Company), were immediately asphyxiated and placed in clean, dry sealable deli cups and brought to the laboratory. In the laboratory, fifteen pairs of the left and right wings from each population were clipped from their bodies and mounted systematically side-by-side, left side-to-right side, on microscope slides which were then covered with another glass slide and glued over to secure the wings. These pairs of wings were then microphotographed and digitized using Leica Microscope DM750 that was coupled to a camera LCC50 and a digitizing software LAS EZ 1.0. The digitized images of *Aedes alsbopictus* wings were then transferred and sorted into folders in a computer. Each of the left and right digitized wing were then plotted with a total of 20 landmarks using tpsDig2 (ver. 2.17)[8] (Table 1). The landmark designations and locations of landmarks were based on Harbach and Knight's Mosquito Taxonomists' Glossary [3]. The locations of the landmarks and corresponding anatomical features of the *A.* wings are shown in figure 1a and 1b. To reduce measurement error, all wings were digitized three times as suggested by Palmer [9].

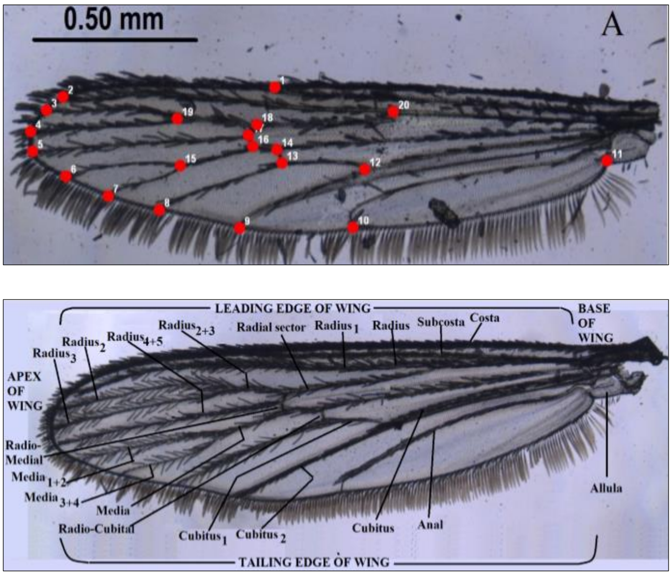

Fig 1 (a) *A. albopictus* wing with 20 landmarks created from TpsDig2 software, and **(b)** labelled generalized mosquito wing showing veins and wing regions

Table 1. Number designations and locations of landmarks used in this study

No.	Location of landmark	No.	Location of landmark
1	Junction of costa and subcostal	11	Axillary incision (distal notch of the alula)
2	Distal end of the first branch of radius	12	Posterior point of the mediocubital crossvein
3	Distal end of the second branch of radius	13	Anterior point of the mediocubital crossvein
4	Distal end of the third branch of radius	14	Forkpoint between M (median vein) and M_{3+4}
5	Distal end of radius $_{4+5}$	15	Fork point of the $M_1$and M_2
6	Distal end of M $_{1+2}$ branch	16	Posterior point of the radiomedial crossvein
7	Distal end of M $_{3+4}$	17	Anterior point of the radiomedial crossvein
8	Distal end of the first branch of the cubitus (Cu1)	18	Forkpoint between R_{2+3} and R_{4+5}
9	Distal end of the second branch of the cubitus (Cu2)	19	Forkpoint of R_2 and R_3
10	Distal end of the anal vein	20	Forkpoint of R_1 and Rs (radius sector)

Prior to analysis with SAGE (Symmetry and Asymmetry of Geometric Data software, ver. 1.04 [10], identification protocol with the numbers of specimens were labeled with the same ID for the replicates in data file (notepad) and saved as specimenidentificationprot.txt. The sides protocol for matching symmetry with "0" as the right side and "1" as the left side in 3 replicates each were first created and saved as sideidentificationprot.txt. Pooled left-right tps files with three replicates in 99 permutations were then loaded and run in SAGE to quantify the wing shape and wing size symmetry in both sexes of the three populations of *Aedes albopictus* tps files. Two-way, mixed model ANOVA table were then constructed with values obtained from the sums of squares, degrees of freedom, means of squares, F and P values for the effects from *individuals, sides, inviduals x sides* and *measurement error* [11]. The *individual's* effect denoted the individual variations of shape and size of *Aedes albopictus* individual mosquitoes. The main effect of *sides* indicated the variation between sides and considered as the measure of directional asymmetry. The *individuals x sides* is the mixed effect, which indicates there is failure of wing shape differences to be on the same side in every case and will therefore indicate there is fluctuating asymmetry in the data. Lastly, *measurement error* is a gauge of the possible effect of measurement error on the estimates of fluctuating asymmetry

1. Results and Discussion

3.1 Wing shape symmetry

For the female populations of *Aedes albopictus,* Procrustes ANOVA showed highly significant variations in symmetry within *individuals* (p<<0.05) and *individuals x sides* (p=0.0<0.05) variations in wing shape but the not on sides for the coastal and poblacion (Table 2). The results indicate that Wing shapes.

the variations in the observed asymmetry for the two populations can be attributed to differences in symmetry among individual mosquitoes. These two populations clearly indicate FA in wing shape but not for the rural population where it showed directional asymmetry (DA). For the males, the poblacion and rural populations showed fluctuating asymmetry (FA) while the coastal population have directional asymmetry (DA) in

Table 2. Procrustes ANOVA of female *Aedes albopictus* for wing shape in matching symmetry**.**

	Effect	SS	DF	MS	F	p
Coastal	Individuals	0.1324	1044	0.0001	1.3578	0******
	Sides	0.0026	36	0.0001	0.7735	0.8298
	Individuals x Sides	0.0975	1044	0.0001	16.7341	0******
	Measurement error	0.0241	4320	0	-	-
Poblacion	Individuals	0.235	1044	0.0002	4.6673	0******
	Sides	0.0017	36	0	0.9892	0.4883
	Individuals x Sides	0.0504	1044	0	16.1882	0******
	Measurement error	0.0129	4320	0	-	-
Rural	Individuals	0.1966	1044	0.0002	3.8445	0******
	Sides	0.00360	36	0.0001	2.044	0.0003***
	Individuals x Sides	0.0511	1044	0	11.9049	0******
	Measurement error	0.0178	4320	0	-	-

Note: sides = directional asymmetry (DA); individual x sides = fluctuating asymmetry (FA); ******p<0.05 = statistically highly significant; ***p<0.05=very significant; ns = statistically not significant (p>0.05); significance was tested with 99 permutations.

Table 3. Procrustes ANOVA of male *Aedes albopictus* for wing shape in matching asymmetry.

	Effect	*SS*	*DF*	*MS*	*F*	*p*
Coastal	*Individuals*	0.2395	1044	0.0002	3.9154	0******
	Sides	0.0073	36	0.0002	3.4455	0******
	Individuals x Sides	0.0612	1044	0.0001	15.3513	0******
	Measurement error	0.0165	4320	0	-	-
Poblacion	*Individuals*	0.2053	1044	0.0002	4.1642	0******
	Sides	0.0008	36	0	0.4498	0.998
	Individuals x Sides	0.0493	1044	0	18.4447	0******
	Measurement error	0.0111	4320	0	-	-
Rural	*Individuals*	0.2758	1044	0.0003	4.4771	0******
	Sides	0.0011	36	0	0.5291	0.9903
	Individuals x Sides	0.0616	1044	0.0001	16.8775	0******
	Measurement error	0.0151	4320	0	-	-

Note: sides = directional asymmetry (DA); individual x sides = fluctuating asymmetry (FA); ******p<0.05 = statistically highly significant; ***p<0.05=very significant; ns = statistically not significant (p>0.05); significance was tested with 99 permutations.

3.2 Wing size variations in *Aedes albopictus*

As for wing size, all populations for both sexes showed fluctuating asymmetry (Table 4 and 5). All the variations in size of the wings are attributed to the variations observed among individuals as shown by the non-significant values attributed to the sides asymmetry (P<1).

Table 4.Procrustes ANOVA of female *Aedes albopictus* for wing size in matching symmetry.

	Effect	*SS*	*DF*	*MS*	*F*	*p*
Coastal	*Individuals*	4199325.2805	29	144804.32	2.764	0.0039***
	Sides	3226.7317	1	3226.7317	0.0616	0.8057
	Individuals x Sides	1519299.3537	29	52389.6329	4638.701	0******
	Measurement error	1355.2837	120	11.294	-	-
Poblacion	*Individuals*	5152683.7949	29	177678.7515	132.0634	0******
	Sides	0.2788	1	0.2788	0.0002	0.9886
	Individuals x Sides	39016.7369	29	1345.4047	172.2227	0******
	Measurement error	937.4407	120	7.812	-	-
Rural	*Individuals*	1130679.8188	29	284160.4765	1000.4436	0******
	Sides	186.9691	1	186.9691	0.6583	0.4238
	Individuals x Sides	8236.9998	29	284.0345	29.058	0******
	Measurement error	1172.9693	120	9.7747	-	-

Note: sides = directional asymmetry (DA); individual x sides = fluctuating asymmetry (FA); ******p<0.05 = statistically highly significant; ***p<0.05=very significant; ns = statistically not significant (p>0.05); significance was tested with 99 permutations.

Table 5. Procrustes ANOVA of male *Aedes albopictus* for wing size characterized by matching symmetry.

	Effect	*SS*	*DF*	*MS*	*F*	*p*
Coastal	*Individuals*	4083736.1786	29	140818.4889	17.4706	0******
	Sides	2976.0973	1	2976.0973	0.3692	0.5482
	Individuals x Sides	233749.0133	29	8060.3108	821.8185	0******
	Measurement error	1176.9476	120	9.8079	-	-
Poblacion	*Individuals*	1400725.391	29	48300.8756	67.8698	0******
	Sides	512.0003	1	512.0003	0.7194	0.4033
	Individuals x Sides	20638.416	29	711.6695	113.7668	0******
	Measurement error	750.6615	120	6.2555	-	-
Rural	*Individuals*	3460670.8825	29	119333.4787	3.2552	0.0011***
	Sides	2162.3808	1	2162.3808	0.059	0.8098
	Individuals x Sides	1063125.2282	29	36659.4906	5620.5682	0******
	Measurement error	782.6858	120	6.5224	-	-

Note: sides = directional asymmetry (DA); individual x sides = fluctuating asymmetry (FA); ******$p<0.05$ = statistically highly significant; ***$p<0.05$=very significant; ns = statistically not significant ($p>0.05$); significance was tested with 99 permutations.

It can be seen in this field study of *Aedes albopictus* at three different areas in Cagayan de Oro city observed FA in all populations based on wing size but not on wing shape where fluctuating asymmetry observed at different populations became progressively mixed with directional asymmetry as shown by DA in the rural female and coastal male populations. It can be argued that this can be considered evidence that stress, as a consequence of habitat disturbance only increases the amount of developmental instability of wing size but not for wing shape which may also change the shape of its mean developmental trajectory as shown by the existence of DA for rural female and coastal male populations. Considering that the terrains of Cagayan de Oro city are progressively being altered because of increasing human activity leading to urbanization, many natural habitats of *Aedes albopictus* may have already been disturbed. An example to these disturbances may include the increasing human population density, the conversion of agricultural fields into tracks of subdivisions for human habitation and the decrease of outdoor water storages due to increasing availability of water supply systems. These alterations may have imposed drastic changes in the habitats of the different populations of *Aedes albopictus*, which may have in turn, put these populations under severe stress. The DA in wing shapes detected in the rural samples of female and coastal male *Aedes albopictus* wings however, could possibly be due to long-term urbanizing effects that the variations in the two populations have a genetic basis on the shape of their wings. Analyses of *Aedes albopictus* wing shape and size with SAGE has demonstrated that asymmetry variations among populations can likely be under the effect of environmental factors, genetic perturbations or both as shown in several studies where a considerable amount of asymmetry have been observed on insect wings [6, 9, 13-22].

4. Conclusions

The results of this study on the symmetry in wing size and shape in *Aedes albopictus* using SAGE indicate that there was developmental instability in wing size since all the three populations have fluctuating asymmetry (FA). These could be attributed to environmental stress the populations of the mosquitoes experienced resulting to asymmetrical sizes of the wings. However for wing shape, the existence of directional asymmetry in two populations – the rural population of the female and coastal population for the male indicate the observed asymmetry in the shape of the wing in *Aedes albopictus* may have a genetic basis. Unlike the other populations, wing shape directional asymmetry in these two populations could have resulted to the long-term urbanizing effects in the city resulting to possible population genetic differentiation in the mosquito.

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