Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 5, p. 109-118, 2015
http://www.innspub.net

INNSPUB

RESEARCH PAPER OPEN ACCESS

Population analysis via fluctuating asymmetry in the wings of *Culex quinquefasciatus* say from selected breeding sites in Iligan City, Philippines

Solaiman M. Tataro, Sharon Rose M. Tabugo*

Department of Biological Sciences, College of Science and Mathematics

Article published on November 11, 2015

Key words: Developmental instability, Fluctuating asymmetry, *C. quinquefasciatus,* Procrustes, ANOVA, SAGE.

Abstract

Fluctuating asymmetry (FA) refers to the unequal bilateral sides of an organism. It occurs when an individual is unable to undergo identical development on both sides of a bilaterally symmetrical trait. It measures the sensitivity of development to a broad array of environmental and genetic stresses. In this study, FA was used to measure developmental instability (DI) the inverse of developmental stability of populations of *C. quinquefasciatus* in (Mahayahay, Tambacan and Tipanoy) Iligan City. Analysis was based on landmark-based Geometric morphometrics (GM) through Procrustes method and makes comparison of FA indices of homologous points and identifies the level of developmental instability. Using landmark based method for shape asymmetry, anatomical landmarks were used and analyzed using Symmetry and Asymmetry in Geometric Data (SAGE) program. Eighteen landmarks were tested for samples for all populations. Procrustes ANOVA results showed variation and highly significant levels of FA for the three populations and no indication of Directional asymmetry (DA). Underlying reasons behind high FA may include stress as experienced by populations (endogenous and exogenous). Significant FA and increase FA present inability of species to buffer stress in its developmental pathways hence, would mean developmental instability and have implications on species fitness, adaptation, quality of individuals and status as a vector. Data obtained on the nature and population status of *C. quinquefasciatus* may help in establishing tailor-made vector control plan for national programmes in the Philippines.

***Corresponding Author:** Sharon Rose M. Tabugo ✉ sharonrose0297@gmail.com

Introduction

Part of the goal of World Health Organization (WHO) Global Programme is to eliminate lymphatic filariasis in various countries in the globe. In this respect, much interest has been devoted to *Culex quinquefasciatus,* because it is known as a vector of filarial nematodes causing diseases. Previous studies, expressed that variation among natural populations of *C. quinquefasciatus*, are associated with different vectorial capacities (Morais *et al*., 2002). Hence, reported vectorial status of *C. quinquefasciatus,* is complex and changing (Pedersen, 2008, Hamon *et al*.,1967), as there are many factors influencing the quality of individuals. Although, lymphatic filariasis transmission is not exclusive to *Culex* as it is said to be transmitted by other species of mosquitoes from four principal genera (*Anopheles, Culex*, *Aedes* and *Mansonia)* and the distribution, ecology, biology and transmission potential of which may vary. Noteworthy, is that transmission efficiency differs considerably by vector species. With this information at hand, it is pertinent to understand the nature of *C. quinquefasciatus* populations found in a specific area (Hamon *et al*.,1967).

There are almost 120 million cases of lymphatic filariasis worldwide and had posed a big problem (Foster and Walker, 2002, Galbo and Tabugo, 2014, Labarthe and Guerrero, 2005, Flores *et al*., 2010). In the Philippines, it is quite alarming that the cases were increasing and spreading. In 2005, there were 645,232 cases reported which 56% of it was from Mindanao. In Iligan City, although, it was not a major outbreak, there were reports from DOH about filarial incidence of residents from Barangay Tipanoy and Tambacan. However, it was not ascertain whether natural populations of *C. quinquefasciatus* from these barangays were really behind the recorded cases. It was also not certain whether patients obtained the infection from these two barangays or outside. Apparently, due to the long incubation period plus growth of the filarial larva inside the body system that is difficult to detect until the onset of visual cues. Hence, assessment of the existing populations of the *C. quinquefasciatus* in the respective areas is important. This led to the investigation of the nature, variation, and developmental status of the species populations found in some major barangays in Iligan.

Previous studies had used fluctuating asymmetry (FA) in population estimates to investigate the nature and status of species in a given area. FA is defined as fine and random deviations from perfect symmetry of organism's morphology. It is known as a reliable factor to detect the developmental stability of an organism because it shows both genetic and environmental stresses and this has been an important theory in evolutionary biology for decades (Palmer, 1994). Thus, it can create an estimate of developmental "noise," that has been utilized as a measure of developmental stability and to evaluate the influence of environmental and genetic stresses on development (Palmer and Strobeck 1986, Waddington, 1942), herewith, a direct relationship between FA and developmental instability (DI). An assumption of FA analysis is that the development of the two sides of a bilaterally symmetrical organism is influenced by identical genes and, therefore, non-directional differences between the sides must be environmental in origin and reflect accidents occurring during development. It increases under both environmental and genetic stress. It reflects a population's state of adaptation, coadaptation and quality (Waddingon, 1942, Graham *et al*., 1993). Overall, increased fluctuating asymmetry is related either to environmental stress or with increased homozygosity; but somehow, exceptions to these relationships are common and few studies directly associate fluctuating asymmetry, selection, fitness and evolutionary change (Merila and Bjorklund, 1995). The ubiquity of symmetry enhanced the idea of having FA as a bioindicator of developmental instability. Also, FA could provide advantages over other bioindicators of stress because FA is cost-effective and easy to measure (Clarke, 1993).

In this regard, this study was done to assess the nature, variation and developmental status of natural populations of *C. quinquefasciatus*. FA as a bioindicator of stress and developmental instability was examined in the wings of *C. quinquefasciatus* collected from selected barangays (Mahayahay, Tambacan and Tipanoy) in Iligan City. The wings were investigated because it has been known to contribute much to the unparalleled success of insects. In the recent years, much interest has also been devoted to the determination and examination of FA as an indicator of individual quality. Here, a hypothesis assumes that fluctuating asymmetry may reflect quality of individuals. Results of FA in the wings may imply the state of adaptation and co-adaptation of *C. quinquefasciatus*. Data obtained may help in establishing tailor-made vector control plan for national programmes in the Philippines.

Materials and methods

Study Area

Iligan city is known as the city of waterfalls. It is geographically within the province of Lanao del Norte. It has a total of land area of 813.37 square kilometers (314.04s mi), making it one of the largest cities in the Philippines in terms of land area.it is bounded on the north by 3 municipalities of Lanao del Norte (Baloi, Linamon and Tagoloan) and the 2 municipalities of Lanao del Sur (Kapai and Tagoloan II), to the northeast by Cagayan de Oro City, to the east by the municipality of Talakag, Bukidnon;and to the west by Iligan Bay. It had a population of 322,821 inhabitants in the (see Fig.1).

Fig. 1. Map of Iligan City, Lanao del Norte, Philippines (left); map showing the 3 barangays (Tipanoy, Mahayahay and Tambacan) that serve as sampling sites (right).

The chosen barangays were Mahayahay, Tamabacan and Tipanoy. They were selected as the sampling sites because these barangays have reported incidence of lymphatic filariasis. Although Brgy. Mahayahay has no incident report but this will be the basis if there is significant difference among the three sites since Brgy. Mahayahay serves as a connecting link between Brgy. Tambacan and Brgy. Tipanoy.

Sample Collection, Processing and Study Procedures

Only female *C. quinquefasciatus* mosquitoes were used in this study because they feed on vertebrate animal blood by sucking for their reproductive cycle. Male *C. quinquefasciatus* mosquitoes feed on plant substance. Sex of the said mosquitoes can be identified through their proboscis, a long needle-like antenna that extends from the area of its mouth. Male mosquitoes have a feather-like proboscis, while the proboscis of female mosquitoes is relatively smooth, not bushy as shown Fig. 2.

Specimens were collected from housing units in Mahayahay, Tambacan and Tipanoy, Iligan City. Collection of adult mosquito samples was done using aerial nets. The adult mosquitoes that were trapped inside the net were transferred into plastic bottles for easy transport to the laboratory. Sampling was done during night time. It was observed that breeding sites include shallow canals, artificial habitats such as drains and drain sumps, wells, stock drinking troughs, septic tanks, rain water containers, tires and various other small containers. The collected adult mosquitoes were sorted and identified in the laboratory using a stereoscope and digital microscope. Only female *C. quinquefasciatus* adults (N=60) per site were utilized in this study. The wings of identified *C. quinquefasciatus*, were gently detached from the thorax using forceps, placed on a glass slide and secured with another glass slide to cover it. Wings were photographed under a dissecting microscope with consistent magnification, and the digital images were kept on file for data analysis.

For morphometric analysis, eighteen (18) anatomical landmarks in the wings of female *C. quinquefasciatus* were used. TPS files were created using TpsUtil and landmarks were digitized using TPSdig2 software. Descriptions of identified landmarks are presented in Table 1. The landmarks were placed at intersections of wing veins or intersections of veins and the wing margin (Fig. 3).

FA levels were measured using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0. This software analyzed the x- and y-coordinates of landmarks per individual. Procrustes superimposition analysis was performed with the original and mirrored configurations simultaneously (Fig. 4). This method removes the effect of location, orientation and scale. The least squares Procrustes consensus of set of landmark configurations and their relabeled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry. The squared average of Procrustes distances for all specimens is the individual contribution to the FA component of variation within a sample. To detect the components of variances and deviations, Procrustes ANOVA was used (Marquez, 2006, Klingenberg and McIntyre, 1998).

Moreover, Principal Component Analysis (PCA) of the covariance matrix associated with the component of FA variation was performed, to carry out an interpolation based on a thin-plate spline to visualize shape changes as landmark displacement in deformation grids (Marquez, 2006) in order to understand variation on respective populations.

Results and discussion

Culex quinquefasciatus Say (Diptera: Culicudae) is a member of the *Culex pipiens* complex. *C. quinquefasciatus,* is often characterized as brown, medium-sized mosquito which is commonly found in households. It is usually found in rural and urban communities in tropical and subtropical regions worldwide. Their breeding system is the same as other mosquitoes, where the male and female mate and then, female lays eggs on water, become larvae and become adult mosquito after many days (Derraik, 2005). Sampling sites from the three barangays were found to be suitable breeding sites of *C. quinquefasciatus* because of presence of bodies of water in the area such as ponds, man-made canals, tires and empty plastic containers. Collection of the specimens was done during the night time since, they are considered as nocturnal opportunistic blood feeders, also to increase the probability of getting *C.quinqefasciatus* over other species because, other species may be also present (e.g. *Anopheles*) (Derraik and Slaney, 2005, Lee *et al.*, 1989).

It was observed that the size of adult female *C. quinquefasciatus* ranged from 3.9 to 4.3 mm. The color of the body was brown. The tarsi, wings, proboscis and the thorax were darker in color than the rest of the body. The abdomen of a female *C. quinquefasciatus* was somewhat bloated than the male. The length of the antennae and the proboscis was the same in length. However, there were cases wherein the antennae is slightly shorter than the proboscis (Galbo and Tabugo, 2014). The flagellum has thirteen segments that have few to no scales

(Lima *et al.*, 2003, Sirivanakam and White, 1987). The thorax scales were narrow and curved. The abdomen was round, narrow and with pale bands on the basal side of the tergite. The bands barely touch the basolateral spots taking on a half-moon shape (Darsie and Ward, 2005). Both male and female depend on plants as a sugar meal. But only female *C. quinquefasciatus* seeks blood for reproduction purposes. They feed on animals such as mammals and birds during night time. Thus, only female *C. quinquefasciatus* was considered for this study.

Table 1. Description of assigned landmarks on *Culex quinquefasciatus* wings.

Landmarks #	Description
1	intersection of costa (C)
2	distal end of the radius (R)
3	radial branch 2
4	radial branch 3
5	distal end of radial branches 4 & 5
6	distal end of M1 & 2
7	distal end of M3 & 4
8	distal end of cubital vein 1
9	distal end of cubital vein 2
10	Anal vein
11	origin of cubital 1
12	midpoint branch of cubital 3
13	medio-cubital cross vein
14	midpoint branch of medial vein
15	radio-sectoral vein
16	radio-medial cross vein
17	midpoint branch of radial vein
18	origin of radius branches 2 & 3

Fluctuating asymmetry of the right and left wings of *C. quinquefasciatus* collected from Mahayahay, Tambacan and Tipanoy were assessed through Procrustes method using SAGE software. FA is directly related to developmental instability (DI). Hence, a common tool in investigating DI. The ubiquity of symmetry is a major advantage of FA over other measures of developmental instability. One could compare developmental instabilities and examine the underlying cause. Here, FA refers to small random deviations from perfect symmetry in bilateral paired structures (i.e. right and left wings) and it is thought to reflect an organism's ability to cope with genetic and environmental stress during development. Its utility as a bioindicator of stress was based on the assumption that perfect symmetry is *a priori* expectation for the ideal state of bilateral structures. It may reflect a population's average state of adaptation and coadaptation (Graham *et al.*, 2010, Parsons, 1990). Also, it is thought to increase under both environment and genetic stress (Graham *et al.*, 2010).

Results of Procrustes ANOVA showed highly significant value of FA in the wings of the specimens from the three sites. FA was determined using the coordinates of the tangential space including the product of the coordinates of the left and right homologous points in formula which provided the final result of the Procrustes ANOVA (Table 2). The mean square of the interaction of "*individuals x sides*" effects revealed a high value compared to the low value of mean square measurement error which indicates highly significant FA for all populations, as also manifested by the high F values for "*individuals x sides*" effect for all sites. However, Tambacan has relatively higher F value compared to the other two sites. A higher F value would mean smaller P value (*P<0.001 is significant). Thus, Tambacan has higher FA level. Only *individual x sides* interaction denotes fluctuating asymmetry (FA). Directional asymmetry (DA) ('sides') was not significant in all samples.

In the light of the results, significant FA and increase FA present inability of species to buffer stress in its developmental pathways hence, would mean developmental instability and have implications on species fitness. Hypothesis assumes that fluctuating asymmetry has costs, reflects the quality of individuals and the level of genetic and environmental stress experienced by individuals or populations during development.

Table 2. Procustes ANOVA results of *C. quinquefasciatus* in three different barangays.

Effects	SS	DF	MS	F	P	Significance
Mahayahay						
Individuals	0.12715	608	0.000209	0.80008	0.99698	ns
Sides	0.00826	32	0.000258	0.98751	0.48872	ns
Individuals x sides	0.15892	608	0.000261	4.7481	0	******
Measurement error	0.14092	2560	5.50E-05	--	--	
Tambacan						
Individuals	0.097304	608	0.00016	0.85041	0.97702	ns
Sides	0.0078617	32	0.000246	1.3055	0.12367	ns
Individuals x sides	0.11442	608	0.000288	4.8843	0	******
Measurement error	0.098636	2560	3.85E-05	--	--	
Tipanoy						
Individuals	0.054123	608	8.9018e-005	0.7482	0.99982	ns
Sides	0.0023278	32	7.2744e-005	0.61142	0.95581	ns
Individuals x sides	0.072338	608	0.00011898	3.774	0	******
Measurement error	0.080706	2560	3.1526e-005	--	--	

Note: side = directional asymmetry; individual x sides interaction = fluctuating asymmetry; * $P < 0.001$, ns – statistically insignificant ($P > 0.05$); significance was tested with 99 permutations, ***** = highly significant.

Based on the results, it suggests that populations of *C. quinquefasciatus* present in these areas have poor developmental homeostasis, thus high developmental instability (DI). Hence, may suggest not fit enough as competent vectors. A recent study by Galbo and Tabugo, 2014 yield the same results for populations from barangay Del Carmen, Palao and San Miguel. The possible cause of developmental instability were well studied and it include a range of environmental factors (e.g. deviant climatic conditions, food deficiency, parasitism, pesticides) and genetic factors (e.g. inbreeding, hybridization, novel mutants) (Mpho *et al.*, 2000).

Table 3. Variance explained by two Principal Component of *C. quinquefasciatus* in three barangays.

Barangay	PC 1(%)	PC2 (%)	Overall (%)
Mahayahay	38	20	58
Tambacan	46	14	60
Tipanoy	38	21	59

In addition, the transmission dynamics of lymphatic filariasis are quite complex, involving two genera of parasite (*Wuchereria* and *Brugia*) and a number of genera of mosquito carriers. The four main genera are: *Anopheles, Culex, Aedes* and *Mansonia*. Thus, the preconceived notion that *Culex* alone was responsible for incidence of filariasis in Iligan may not be true because other mosquitoes could be involved locally. This is in line with reports where lymphatic filariasis was transmitted mainly by *Anopheles* mosquitoes. *Anopheles* and *Culex* bite predominantly at night, and a number of anopheline species prefer to bite humans and to rest indoors. The transmission of lymphatic filariasis parasites is considered to be less efficient than that of other vector-borne parasites, such as malaria and dengue, because the rate of uptake of microfilariae by a mosquito vector from a human host depends on the prevalence and intensity of infection in the community and the biting rate of the mosquito. In general, the greater the number of infectious hosts available in a community with a moderate-to-high density of circulating microfilariae in their peripheral blood and the higher the biting rate, the higher the chance of a mosquito picking up microfilariae from a human host and causing transmission.

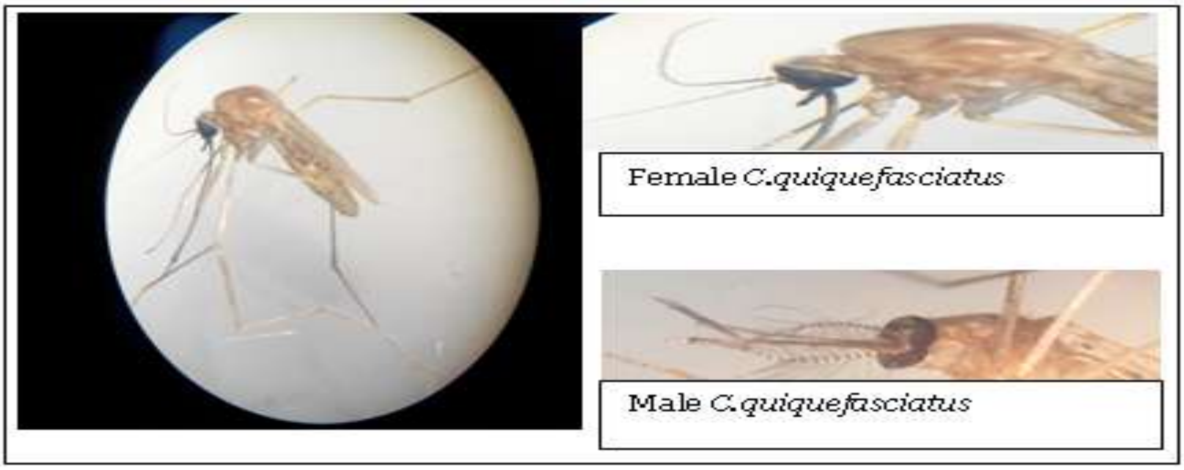

Fig. 2. *Culex quinquefasciatus* mosquito female (left); difference between male and female (right) see proboscis.

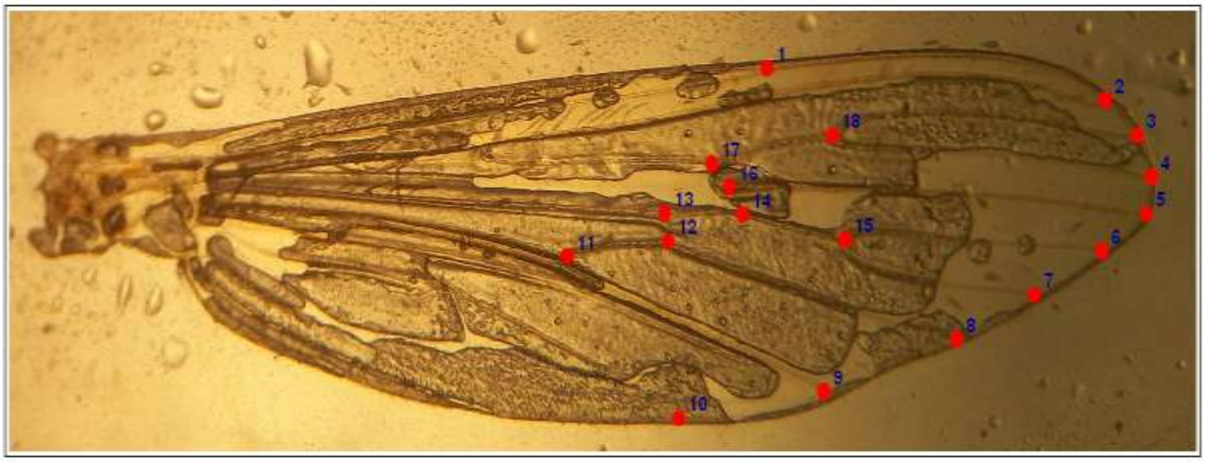

Fig. 3. Location of eighteen landmarks in the wing of *C. quinquefasciatus*.

Extremely high levels of microfilariae in the blood may, however, result in a substantial number of mosquito deaths as the larvae develop. It was also noted that thresholds are likely to vary in different areas because of the heterogeneity of the vector–parasite relationship. Local environmental conditions affect nature populations and also transmission. This include rainfall, temperature, humidity and soil type can all affect the production of breeding sites and the survival of adult mosquitoes (Foster and Walker, 2002, Labarthe and Guerrero, 2005).

Moreover, Principal Component Analysis (PCA) was also performed in order to visualize the covariance shape change for each principal component and to see the general direction and magnitude of the fluctuation for each landmark. The red dots represent the morphological landmarks used in the study while the blue arrows indicate the direction as well as the magnitude of the fluctuation. The percentage values of PCA represent the level of variability in the data. Here, the amount of overall variation exhibited by PC1 and PC2 of samples from Tambacan exhibited more percentage of variation than samples from Mahayahay and Tipanoy (Table 3 and Fig. 5), although the values are quite close. Also, higher FA was exhibited by the samples from Tambacan.

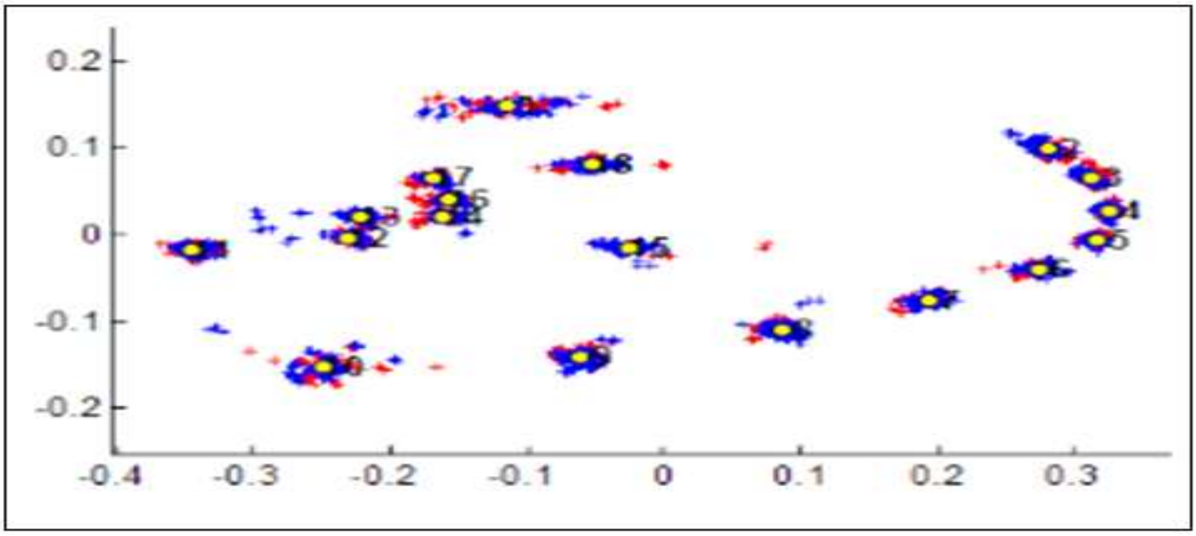

Fig. 4. Procrustes fit of right (red) and left (blue) sides data of wings.

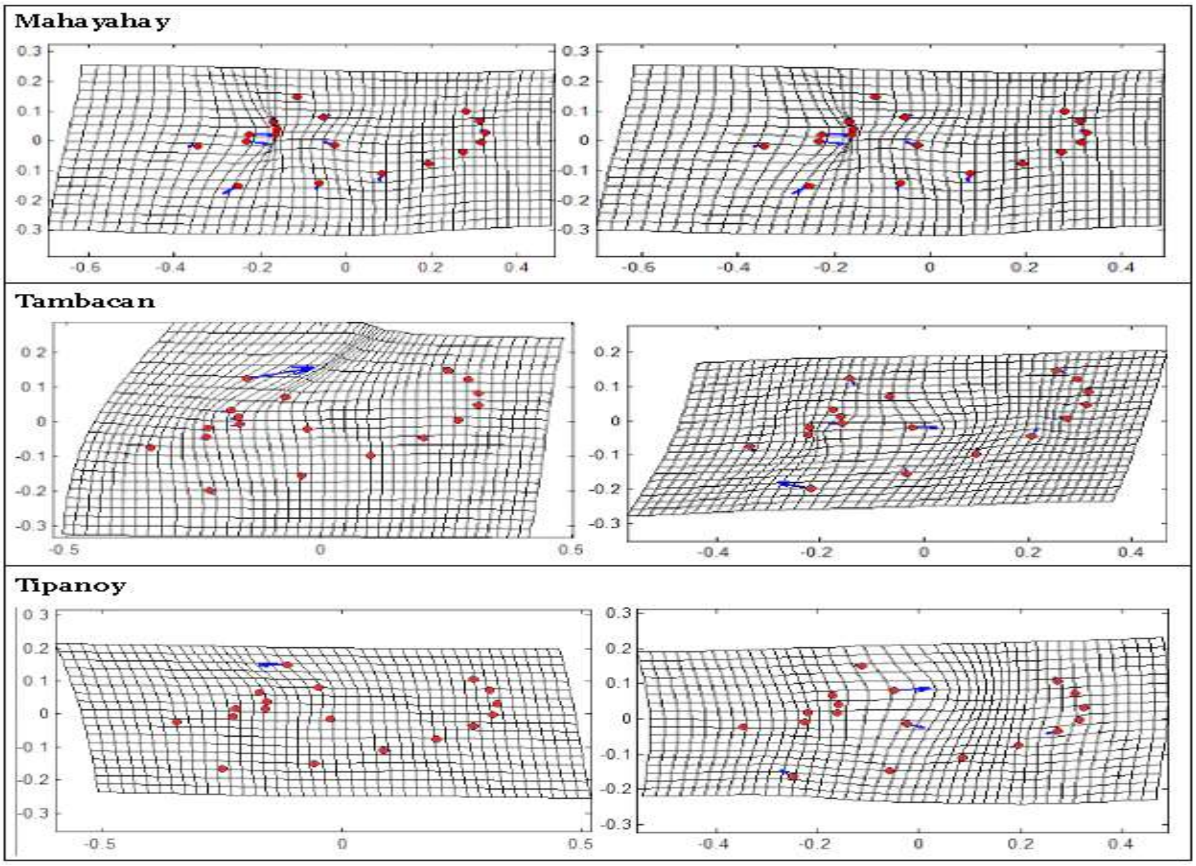

Fig. 5. PCA implied deformation for individual x side interaction of fluctuating asymmetry C. quinquefasciatus in the three barangays where, more variation were exhibited by Tambacan samples (see magnitude of blue arrows).

Noteworthy is that, significant FA and increase FA present inability of species to buffer stress in its developmental pathways hence, would mean developmental instability and have implications on species fitness, adaptation, quality of individuals and status as a vector.

This may suggest that *C. quinquefasciatus* populations are developmentally unstable and could be due to genetic and environmental stress. Hence, further suggests that populations may not be fit enough as competent vectors and may be easily eradicated if correct measures are implemented.

Conclusion

Developmental stability is the ability of an organism to control its development against genetic and environmental conditions and produce a genetically known phenotype. This study used landmark-based Geometric Morphometrics (GM) and Procrustes ANOVA to measure the fluctuating asymmetry (FA), a measure of developmental instability (DI). There is inverse relationship between developmental stability and FA. Results showed that *C.quinquefasciatus* found in Barangay: Mahayahay, Tambacan, and Tipanoy have high FA, thus high DI.

This may suggest that *C. quinquefasciatus* populations are developmentally unstable and could be due to genetic and environmental stress. Hence, may suggest not fit enough as competent vectors. The preconceived notion that *Culex* alone was responsible for incidence of filariasis in Iligan may not be true because other mosquitoes may be involved locally. However, further investigation is still required for other populations from different barangays. This study assessed the potential for FA as a bioindicator of stress and determined developmental instability in the wings of *C. quinquefasciatus* collected from selected barangays (Mahayahay, Tambacan and Tipanoy) in Iligan City. Data obtained on the nature and population status of *C. quinquefasciatus* may help in establishing tailor-made vector control plan for national programmes in the Philippines.

Acknowledgment

The researchers would like to express their heartfelt gratitude to their families and friends who become a considerable source of inspiration and determination.

References

Clarke GM. 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. Environmental Pollution **82,** 207–211.

Darsie Jr RF, Ward RA. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. Gainesville, FL: University of Florida Press, 300.

Derraik JGB. 2005. Mosquitoes breeding in phytotelmata in native forests in the Wellington region, New Zealand. New Zealand Journal of Ecology **29(2),** 185-191.

Derraik JGB, Slaney D. 2005**.** Container aperture size and nutrient preferences of mosquitos (Diptera: Culicidae) in the Auckland region, New Zealand. Journal of Vector Ecology **30 (1)**, 73-81.

Flores FS, Diaz LA, Batalla´n GP, Almiro´n WP, Contigiani MS. 2010**.** Vertical transmission of St. Louis encephalitis virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Co´rdoba, Argentina. Vector Borne Zoonotic Dis **10,** 1002.

Foster WA, Walker ED. 2002**.** Mosquitoes (Culicidae). In: Mullen G, Durden L. (editors). Medical and Veterinary Entomology. New York, NY: Academic Press, 245-249.

Galbo KR, Tabugo SRM. 2014**.** Fluctuating asymmetry in the wings of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) from selected barangays in Iligan City, Philippines. AACL Bioflux **7(5),** 357-364.

Graham JH, Raz S, Hagit H, Nevo E. 2010**.** Fluctuating Asymmetry: Methods, Theory and

Applications. Symmetry **2,** 466-495.

Graham JH, Freeman DC, Emlen JM. 1993**.** Developmental stability: A sensitive indicator of populations under stress. In: Landis, WG; Hughes, JS; Lewis MA (eds.). Environmental Toxicology and Risk Assessment, ASTM STP, Philadelphia, PA: American Society for Testing Materials, 1179.

Hamon J, Burnett GF, Adam JP, Rickenbach A, Grjebine A. 1967**.** *Culex pipiens* fatigans Wiedeman, *Wuchereria bancrofti* Cobbold, et le developpement economique de l' Afrique tropicale. Bulletin WHO, 207-237.

Labarthe N, Guerrero J. 2005**.** Epidemiology of heartworm: what is happening in South America and Mexico. Vet. Parasitol **133,** 149–156.

Klingenberg CP, McIntyre GS. 1998**.** Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution **52,** 1363–1375.

Lee DJ, Hicks MM, Debenham ML, Griffiths M, Marks EN, Bryan JH, Russell RC. 1989**.** The Culicidae of the Australian region. Canberra, Australia: Australian Government Publishing Service **7,** 281.

Lima CA, Almeida WR, Hurd H, Albuquerque CM. 2003**.** Reproductive aspects of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wuchereria bancrofti* (Spirurida: Onchocercidae). Memórias do Instituto Oswaldo Cruz **98,** 217-222.

Marquez E. 2006. Sage: symmetry and asymmetry in geometric data. Ver 1.04. Michigan: University of Michigan Museum of Zoology.

Merila J, Bjorklund M. 1995**.** Fluctuating asymmetry and measurement error. Syst. Biol. **44,** 97-101.

Morais SA, Moratore C, Suesdek L, Marrelli MT. 2002**.** Genetic morphometric variation in *C. quinquefasciatus* from Brazil and La Plata. Memórias do Instituto Oswaldo Cruz **97(8),** 1191-1195.

Mpho M, Holloway GJ, Callaghan A. 2000. The effect of larval density on life history and wing asymmetry in the mosquito *Culex pipiens.* Bulletin of Entomological Research **90,** 279-283.

Palmer AC, Strobeck C. 1986**.** Fluctuating asymmetry - measurement, analysis, patterns. Annual Review of Ecology and Systematics **17,** 391-421.

Palmer RA. 1994. Fluctuating asymmetry analysis: a primer. In: MarkowTA (ed.) Developmental Instability:Its Origins and Evolutionary Implications. London: Kluwer Academic.

Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biol. Rev. **65,** 131–145.

Pedersen EM. 2008. Vectors of lymphatic filariasis in Eastern and Southern Africa and the prospect for supplementary vector control. Bagamoyo Tanzania **13,** 1107.

Sirivanakarn S, White GB. 1978**.** Neotype designation of *Culex quinquefasciatus* Say (Diptera: Culicidae). Proceedings of the Entomological Society of Washington **80,** 360-372.

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters.