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Determining Developmental Instability via Fluctuating Asymmetry in the Shell Shape of *Arctica islandica* Linn. 1767 (ocean quahog)

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ABSTRACT

Developmental stability is the ability of organisms to maintain a stable state despite different environmental conditions and stressors. The converse of which is developmental instability where, one can estimate by subjecting genetically identical individuals in a common environment and measuring fluctuating asymmetry (FA). This type of asymmetry examines random variation between left and right sides of traits that are on average, bilaterally symmetrical since, both sides of a symmetrical structure are said to be genetically identical, with similar history of gene activity and experiencing the same environment. Fluctuating asymmetry (FA) is the most commonly used tool for measuring developmental instability herewith, a direct relationship between FA and developmental instability. In this study, a population of the bivalve Arctica islandica (ocean quahog) was analyzed for fluctuating asymmetry. The population consists of 83 individuals and 3 different morphotypes were identified, FA of each morphotype was measured to evaluate developmental stability. Hypothesis assumes that fluctuating asymmetry has costs and reflects the quality of individuals. Using landmark method for shape asymmetry, anatomical and mathematical landmarks were assigned in the inner valves of Arctica islandica (ocean quahog) and analysis was done using "Symmetry and Asymmetry in Geometric Data" (SAGE) program. Results yield highly significant FA in Morphotye C, significant FA in morphotype A and non-significant FA in Morphotype B. Results implied that developmental homeostasis seems not easily disturbed in Morphotype B hence, a quite stable morphotype. Thus, suggesting stabilizing selection may be at work.

INTRODUCTION

Developmental stability is defined as the ability of an organism to moderate its development against genetic or environmental conditions and produce the genetically determined phenotype [1]. Developmental stability is influenced by both genotype and environment, as evidenced by different genotypes displaying different levels of stability under the same environmental condition; and identical genotypes displaying different levels of stability under varying environments. Fluctuating asymmetry (FA) is the most commonly used tool for measuring developmental instability herewith, a direct relationship between FA and developmental instability [1,2].

Symmetry is everywhere in the living world. Many types of symmetries including bilateral, radial, rotational, dihedral, and translational symmetries are present in all major groups of organisms [3,4,5,6]. There are three types of biological asymmetry, fluctuating asymmetry, directional asymmetry, and antisymmetry. Fluctuating asymmetry is characterized by small random deviations from perfect bilateral symmetry. These small random deviations result in a normal or leptokurtic distribution of asymmetry around a mean of zero. Directional asymmetry is characterized by a symmetry distribution that is not centred around zero but is biased significantly, towards larger traits either on

the left or the right side. Antisymmetry is characterized by being centred around a mean of zero. Directional symmetry and antisymmetry are developmentally controlled and therefore likely to have adaptive significance while fluctuating asymmetry is not likely to be adaptive as symmetry is expected to be the ideal state [4,7,8]. Because all life forms are more or less symmetrical, fluctuating asymmetry may be observed in all taxa [9]. The ubiquity of symmetry is a major advantage of fluctuating asymmetry over other measures of developmental instability. One could conceivably compare developmental instabilities of invertebrates and vertebrates and attempt to decipher the underlying causal stress [9]. The underlying assumption of fluctuating asymmetry 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 [1].

Fluctuating asymmetry are fine and random deviations from perfect symmetry of organism's morphology. It is considered a reliable factor for measuring developmental instability because it reflects both genetic and environmental stresses and this has been an important theory in evolutionary biology for decades [10]. Fluctuating asymmetry is important because it reflects a population's state of adaptation and coadaptation. Moreover, it increases under both environmental and genetic stress though responses may be inconsistent [9]. Deviations from perfect symmetry may be measured as variances of linear dimensions, shape variation involving landmarks, or as continuous symmetry measures.

The measurement of fluctuating asymmetry is complicated by the fact that its magnitude and distribution are the same as the magnitude and distribution of measurement error. Therefore, in order to establish that real differences in symmetry rather than just measurement error being reported, it is imperative to establish that the measures of fluctuating asymmetry explain a statistically significant proportion of the observed total variance between the sides. To achieve this it is necessary to make repeated measures of the left and right sides of the trait. The repeated measures need to be made on the same subjects in ignorance of the initial measure, with the same equipment and under the same laboratory or field circumstances as those of the main data. To eliminate bias, ideally all measurements should be made in ignorance of the measurement recorded for the side's pair. For analysis of a potential fluctuating asymmetry data set, certain criteria must be met: the measurements must represent actual deviations from symmetry and not measurement error, and the distribution of fluctuating asymmetry must conform to that expected for it, rather than for directional asymmetry or antisymmetry [7].

Hence, in this study, the software used was "Symmetry and Asymmetry in Geometric Data" SAGE, to determine whether fluctuating asymmetry is significantly different from measurement error. Data obtained were analyzed between groups, in a population by using a method for comparing trait variances. In this study FA was investigated for *Arctica islandica* (ocean quahog), a species of edible clam, and a marine bivalve mollusk in the family Arcticidae. This species is commercially harvested as an important food source in Iligan City. This species are also known by a number of different common names, including Icelandic cyprine, mahogany clam, mahogany quahog, black quahog, and black clam [11]. Species are often buried in the sediment of the seabed, where they are safe from predation while others lie on the sea floor or attached to rocks or other hard surfaces thus, can also be used as bioindicators [12]. The typical *Arctica islandica* resembles the quahog, but the shell of the ocean quahog is rounded, the periostracum is usually black, and on the interior of the shell, the pallial line has no indentation, or sinus. Unlike the quahog which lives intertidally and can be collected by clam digging, this species lives subtidally, and can only be collected by dredging. They grow to sizes exceeding 50 mm shell height [13].

The study aims to determine developmental instability via fluctuating asymmetry of the bivalve species *Arctica islandica.* Specifically, it aims to investigate differences in the fluctuating asymmetry (right and left valves) of three different morphotypes in one population.

Recently, much interest has also has been devoted to the determination and examination of FA as an indicator of individual quality. Here, a hypothesis assumes that fluctuating asymmetry has costs and reflects the quality of individuals. Hence, this study may be able to generate knowledge and provide information on the nature of the organism also which morphotype is likely dominant and relatively developmentally stable hence, can be cultivated to maximize yield or food production.

MATERIALS AND METHODS

1. Sample Processing

Samples were collected and bought from Palao, Iligan City. Three (3) morphotypes were identified from one population (Table 1). The samples were cleaned, and then soft tissues were removed. Photographs of the inner right and left valves were used for testing fluctuating symmetry. Total population size was 83 individuals consisting of 31 individuals for morphotype A, 39 individuals for morphotype B, and 13 individuals for morphotype C.

2. Landmark Assignment

Anatomical and mathematical landmarks were assigned in the inner valves of *Arctica islandica* (ocean quahog). Thirteen (13) landmark points were used, composed of 8 anatomical landmarks and 5 mathematical landmarks (Figure 1 and Table 2). Anatomical landmarks, the most biologically informative, are points that are biologically homologous between organisms. Mathematical landmarks, generally less informative than anatomical landmarks, are defined by some mathematical or geometric property such as points of maximum curvature or extreme points [14]. Analysis was based on definite and repeatable anatomical marks on the interior of the valves.

Table 1. Different morphotypes in the population.

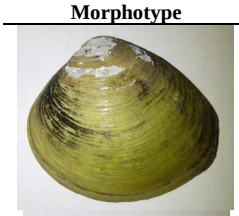
Morphotype	Description
	**Morphotype A** is light yellow to brown and have moderate amount of black streaks. White blotches near the umbo are eroded calcium carbonate from the valves.
	Morphotype B has darker color than morphotype A- dark yellowish. There are also black streaks distributed unevenly through the exterior valve.
	Morphotype C has the darkest colors; brown to black white blotches near umbo are also present as they are the eroded area of the valve.

Figure 1. Location of the 13 landmarks on bivalve interior of *Arctica islandica*

Table 2. Position of the thirteen landmarks selected in the interior valve of *Arctica islandica.*

Landmark #	Position
	Anatomical Landmarks
1	Umbo
2	End of ligament
3	Junction of posterior retractor and posterior adductor
4	Junction of posterior adductor and pallial sinus
5	Inside of pallial sinus
6	Outside of pallial sinus
7	Junction of anterior adductor and pallial line
8	Junction of anterior retractor and anterior adductor
	Mathematical Landmarks
9	Near umbo
10	Dorsal margin maxima
11	Posterior margin maxima
12	Ventral margin maxima
13	Anterior margin maxima

The right and left valves were oriented in the horizontal plane and then the anterior and posterior retractor landmarks were rotated to a horizontal line. The interior of one valve per specimen was then digitally imaged and landmarked using TPS software. *Arctica islandica* valves are symmetrical, allowing the database to be maximized by using both right valves and digitally-mirrored left valves using Microsoft Office Picture Manager.

3. Measurement of Fluctuating Asymmetry

There are three types of deviation from perfect bilateral symmetry: fluctuating asymmetry (FA), directional asymmetry (DA), and antisymmetry (AS). FA measures the variance in left-right (L-R) differences, which are distributed around 0. FA corresponds to a random variation and can be used to measure developmental instability, whereas DA and AS are considered to be inappropriate as descriptors of developmental stability because both are developmentally controlled and are probably adaptive as asymmetries.

The levels of FA were obtained using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0 [15]. This software analyzed the x- and y-coordinates of landmarks per individual. Sides (directional asymmetry; DA), Individual x sides (fluctuating asymmetry; FA), and their respective error were included as effects. Procrustes superimposition analysis was performed with the original and mirrored configurations simultaneously using the SAGE program. This software analyzed the coordinates of the landmarks per individual, using a configuration protocol for both right and left valves. The least squares Procrustes consensus of set of landmark configurations and their relabelled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry [15, 16]. 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, a Procrustes ANOVA was used. The ANOVA used most frequently for fluctuating asymmetry is a two-way, mixed-model ANOVA with replication. The main fixed effect is *sides* (*S*), which has two levels (left and right). The block effect is *individuals* (*I*), which is a random sample of individuals from a population. The *sides by individuals interaction* (*S x I*) is a mixed effect. Finally, an error term (*m*) represents measurement error (replications within *sides by individuals*). The effect called *sides* is the variation between the two sides; it is a measure of directional asymmetry. The effect called *individuals* is the variation among individual genotypes; the *individuals* mean square is a measure of total phenotypic variation and it is random. Meanwhile, the *individual by sides interaction* is the failure of the effect of individuals to be the same from side to side. It is a measure of fluctuating asymmetry and antisymmetry thus, a mixed effect. The error term is the measurement, and is a random effect [17,18, 19]. Principal Component Analysis (PCA), of the covariance matrix associated with the component of FA variation were also performed for each morphotype to carry out an interpolation based on a thin-plate spline to visualize shape changes as landmark displacement in the deformation grid [15,20].

RESULTS AND DISCUSSION

In this study, developmental instability was investigated via fluctuating asymmetry of the bivalve species *Arctica islandica* (ocean quahog). It specifically looked into differences in the fluctuating asymmetry (FA) of the right and left valves for three different morphotypes in one population. The analysis was based on the Procrustes method.

Procrustes ANOVA results yield significant FA in the valves of the specimens classified under morphotype A and C (Table 3).

Table 3. Procrustes ANOVA Results for the three morphotypes of *Arctica islandica* (ocean quahog).

Effects	SS	dF	MS	F	Remarks
Morphotype A					
Sides	0.0012902	22	5.8644e-005	0.36418	
Individuals x sides	0.10628	660	0.00016103	1.1175*	Significant
Measurement Error	0.19654	1364	0.00014409	--	
Morphotype B					
Sides	0.00099396	22	4.518e-005	0.16138	
Individuals x sides	0.23405	836	0.00027997	0.91927 ns	Non significant
Measurement Error	0.52262	1716	0.00030456	--	
Morphotype C					
Sides	0.0009549	22	4.3405e-005	0.20331	
Individuals x sides	0.056361	264	0.00021349	1.886 ******	Highly Significant
Measurement Error	0.064726	572	0.00011316	--	

*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.*

The interaction of both factors side and individuals showed a high value of mean square and a low value of mean square measurement error. Thus, the F value suggested significant FA for individuals in morphotype A and C. Morphotype C showed highly significant FA, based on the F value obtained. Morphotype A showed also significant FA value while Morphotype B had a non-significant FA. In this regard, fluctuating asymmetry refers to small random deviations from perfect symmetry in bilaterally paired structures (i.e. right and left valves), it is thought to reflect an organism's ability to cope with genetic and environmental stress during development and its utility as an indicator of such stresses is based on the assumption that perfect symmetry is an a priori expectation for the ideal state of bilateral structures. Here, fluctuating asymmetry may be used as an indicator of individual quality in studies of natural and sexual selection and as a bioindicator tool for environmental monitoring and conservation biology [21]. A high significant FA would mean more developmentally unstable phenotype.

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 (Table 4 and Figure 2). Here, the amount of overall variation exhibited by PC1 and PC2 of Morphotype B was found to be intermediate of Morphotype A and C, with morphotype C exhibiting the greatest percentage of variation. Higher FA was also observed for morphotype C.

Table 4. Variance explained by first two principal components between the three morphotypes of *Arctica islandica* (ocean quahog).

Morphotype	PC 1 (%)	PC 2 (%)	Overall (%)
A	23	18	41
B	27	17	44
C	31	28	59

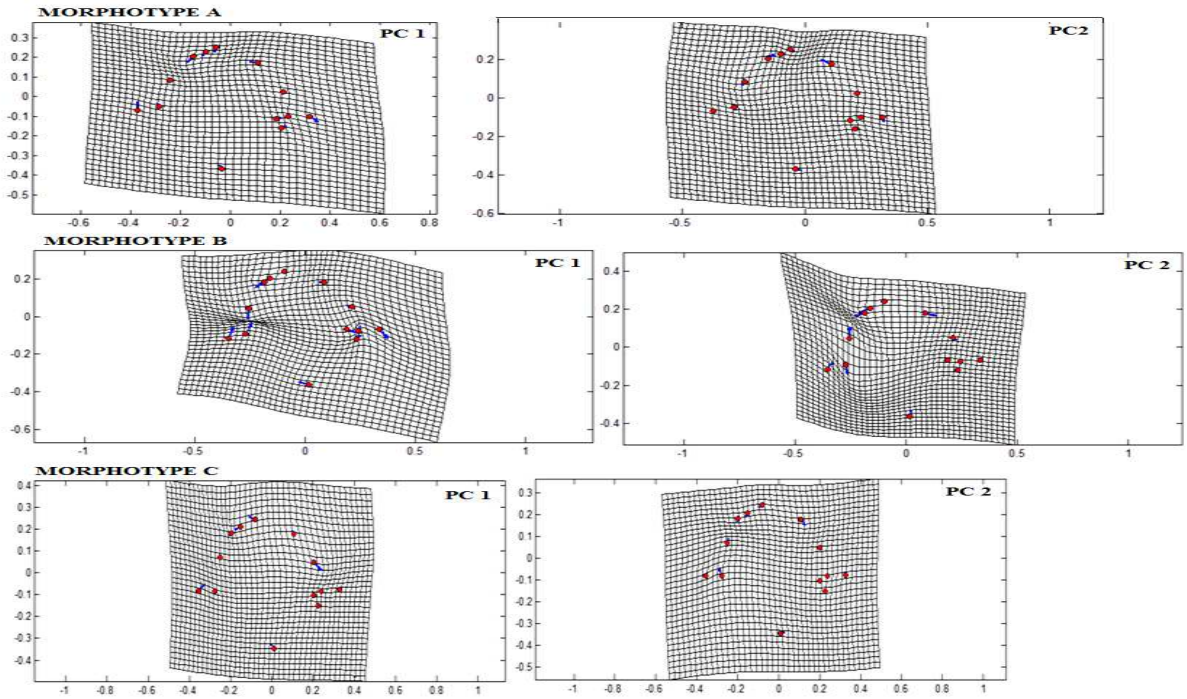


Figure 2. PCA implied deformation for individual x side interaction of fluctuating asymmetry of morphotype A, B and C of *Arctica islandica.*

In the light of the results, it somehow, suggested the role of natural selection. Such that it can alter the frequency distribution of heritable traits in three ways, depending on which phenotypes in a population are favored. The three modes of selection are called directional, disruptive, and stabilizing selection. Directional selection is most common during periods of environmental change or when members of a population migrate to a new habitat with different environmental conditions. Directional selection shifts the frequency curve for a phenotypic character in one direction by favoring individuals who deviate from the average. Meanwhile, disruptive selection occurs when environmental conditions favor individuals at both extremes of the phenotypic range over those with intermediate phenotypes. Moreover, stabilizing selection favors intermediate variants and acts against extreme phenotypes. Stabilizing selection reduces variation and maintains the status quo for a trait [22]. In this case stabilizing selection may be at work where, Morphotype B (intermediate variant) yields non-significant FA results. Hence, suggests Morphotype B as seemingly favorable such that stressors from the environment may not disturb developmental homeostasis. Possible reasons are: (1) the maintenance of genetic diversity in natural populations has been accounted to the fitness benefit of heterozygotes [23] [24]. The underlying reason for an expectation of such positive association between heterozygosity and fitness is that heterozygotes have been suggested to be buffered against environmental variation and thus to be phenotypically plastic i.e. maintain the optimal phenotype in the face of environmental fluctuation. In other words, individuals with relatively high levels of heterozygosity may have higher fitness because they also show a relatively high level of individual homeostasis or stability. However, the literature on the association of heterozygosity and fitness is at best suggestive [25]; (2) Coadapted gene complexes may evolve in a population if natural selection favours alleles in several loci that interact to produce developmentally stable phenotypes [26]. Results show that morphotype A and C exhibit high FA and thereby, considered relatively unstable morphotypes and indicative that it can be experiencing more environmental and genetic stress and has inability to buffer such stress. Meanwhile, morphotype B is likely dominant and relatively developmentally stable hence, can be cultivated to maximize yield or food production.

CONCLUSION

Fluctuating asymmetry is defined as deviations from symmetry which may be caused by environmental stresses, developmental instability and genetic problems during development. It is thought that the more perfectly

symmetrical an organism is, the better it has been able to handle developmental stress and has more developmental stability. Fluctuating asymmetry (FA), then, may be a measure of good-genes that is difficult or impossible to mask. Significant values for Fluctuating asymmetry were observed in Morphotype A and C suggesting deviations in its bilateral symmetry thus, indicate developmental instability. Meanwhile, morphotype B of the population had the least or non significant value for FA, thus Morphotype B is seemingly a favorable phenotype such that stressors from the environment may not disturb developmental homeostasis hence, suggesting higher developmental stability. Also it was observed that more number of individuals in the population has morphotype B, suggesting its relatively higher rate for survival and adaptation to its environment.

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REFERENCES

[1] CH Waddington. *Nature*, **1942**, 150, 563-565.
[2] VM Zakharov. *Future Prospects for Population Phenogenetics*, **1989**.
[3] ER Abraham. *Mar. Biol.*, **2001**, 138, 503-510.
[4] V Valen. *Evolution*, **1962**, 16, 125-142.
[6] VM Zakharov; JH Graham. *Acta zoologica Fennica*, **1992**, 191, 1-200.
[7] AR Palmer. Fluctuating asymmetry analysis: a primer. In Developmental Instability: Its Origins and Evolutionary Implications, [Markow, TA (ed.)], Kluwer Academic, London, **1994**.
[8] SW Gangestad; R Thornhill. *Journal of Evolutionary Biology*, **1999**, 12, 402-416.
[9] JH Graham; DC Freeman; JM Emlen. Developmental stability: A sensitive indicator of populations under stress, In Environmental Toxicology and Risk Assessment, ASTM STP 1179, [Landis, WG; Hughes, JS; Lewis MA (eds.)], American Society for Testing Materials, Philadelphia, PA, **1993**.
[10] PA Parsons. *Biol. Rev. Camb. Philos.* Soc., **1990**, 65(2), 131-45.
[11] M Sabatini; PF Pizzarola, accessed February 26, **2014** httpp://www.marlin.ac.uk/species/Arcticaislandica.htm.
[12] PA Jell. *Alcheringa an Australasian Journal of Palaeontology*, **1980**, 4 (3): 233–239.
[13] R Witbarrd; MJN Bergman. *Journal of Sea Research*, **2003**, 50: 11–25.
[14] IL Dryden; KV Mardia. Statistical Shape Analysis. J. Wiley, New York, **1998**.
[15] E Marquez. **2006** Sage: symmetry and asymmetry in geometric data. Ver 1.0.
http://www.personal.umich.edu/~emarquez/morph/. HK Pfau. *Adv. Odonatol.*, **1991**, 5, 109-141.
[16] CP Klingenberg; GS McIntyre and SD Zaklan. Proceedings of the Royal Society of London B, Biological Sciences, **1998**; 265, pp.1255–1259.
[17] ML Samuels; G Casella; GP McCabe. *J. Am. Stat. Assoc*., **1991**, 86, 798-808.
[18] AR Palmer; C Strobeck. *Annu. Rev. Ecol. Syst.*, **1986**,17,391-421.
[19] AR Palmer; C Strobeck. Fluctuating asymmetry analyses revisited. In Developmental Instability: Causes and Consequences, [Polak, M. (ed.)], Oxford University Press. New York, USA, **2003**.
[20] AL Albarra-Lara; L Mendoza-Cuenca; S Valencia-Avalos; A Gonzalez-Rodrıguez. *Int J Plant Sci.*, **2010**, 171(3):310–322.
[21] J Tomkins; J Kotiaho. Fluctuating Assymetry. Encyclopedia of Life Sciences, **2001**.
[22] N Campbell; J Reece. Biology: Cummings, **2005** chapter 23, p. 480-481.
[23]JB Mitton; MC Grant. *Ann. Rev. Ecol. Syst.*, **1984**, 15: 479-499.
[24] D Charlesworth; B Charlesworth. *Annual Review of Ecology and Systematics*, **1987**, 18: 237–268.
[25]HB Britten. *Evolution*, **1996**, 50: 2158–2164.
[26] JH Graham JH. *Acta Zoologica Fennica*, **1992**, 191: 121–131.