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Fluctuating asymmetry as an indicator of ecological stress and developmental instability of *Gafrarium tumidum* (ribbed venus clam) from Maak and Lagoon Camiguin Island, Philippines

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Abstract. Fluctuating asymmetry (FA) 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. FA increases as the environmental stress increases and thus referred to as a tool for measuring developmental stability and environment quality. This study demonstrates the use of FA as a tool for monitoring developmental stability of *Gafrarium tumidum*. The purpose of this study was to quantify and compare fluctuating asymmetry (FA) of *G. tumidum* from two different sites of Camiguin Island. Analysis was based on the Procrustes method and makes comparison of index of FA of homologous points and identifies the level of developmental stability of the bivalve species. Using landmark method for shape asymmetry, anatomical and mathematical landmarks were used and analyzed using Symmetry and Asymmetry in Geometric Data (SAGE) program. Procrustes ANOVA results showed significant evidence of FA for both populations. Possible explanation for high levels of FA detected may rise from the differences in genetic composition of the populations resulting in different tolerance to stress. Also the presence of environmental disturbances may also lead to increased level of FA. This implies that species present in the two areas exhibit inability to buffer stress in its developmental pathways under different environmental conditions and manifest it as increase level of FA hence, have possible implications on species fitness and adaptation.
Key Words: Morphology deviation, genetic stress, environmental stress, measuring tool, bivalve specie.

Introduction. Developmental stability refers to an organism's ability to buffer environmental and genetic perturbations experienced during its ontogeny (Waddington 1942; Zakharov 1989; Clarke 1998). It is assumed that it is influenced by genotype, environment and/or genotype by environment interactions (Van Dongen & Lens 2000). The small and random departures from perfect symmetry in an organism's bilateral traits are described as fluctuating asymmetry (FA). FA appears to be taxon, trait and stress specific, indicating that environmental noise has a different impact on developing organs and organisms (David et al 1998; Roy & Stanton 1999; Vollestad et al 1999; Bjorksten et al 2001). It is also suggested that increased asymmetry reflects an increased inability to cope with stressful situations (Palmer & Stobeck 1986; Pomiankowski 1997). It is reasonable to expect that, in natural situations, the relationship between FA and stress would be even less consistent and reliable (Rasmuson 2002). However, the use of FA as an indicator of developmental stability and a measure of ecological stress is based on the assumption that a stressful environment would result in higher FA levels than those observed in optimum environments (Velickovic 2004; Parsons 1961, 1962, 1990, 1992; Van Valen 1962; Palmer & Strobeck 1986; Leong et al 2013). This assumption would be supported if there were a consistent level of developmental stability of traits across populations. A consistent developmental stability would give rise to a similar level of FA under the same conditions in all populations. Nevertheless, in the field a number of uncontrolled factors can confound the relationship between stress and FA.

Moreover, one could conceivably compare developmental instabilities of invertebrates and vertebrates and attempt to decipher the underlying causal stress. FA is the most commonly used tool for measuring developmental instability herewith, a direct relationship between FA and developmental instability. An underlying 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 is noted that FA is important because it reflects a population's state of adaptation, coadaptation, fitness and reflects individual quality. It increases under both environmental and genetic stress (Waddington 1942; Graham et al 1993). Both genomic and environmental changes can increase FA which represents a possible deterioration in developmental homeostasis apparent in adult morphology. Genetic perturbations include intense directional selection and certain specific genes. While, environmental perturbations include temperature extremes in particular, protein deprivation, audiogenic stress, and exposure to pollutants (Mpho et al 2000).

Gafrarium tumidum, is a common species of bivalves that burrow shallowly in muddy sand near mangroves. It is small sturdy clam with a ribbed pattern is sometimes seen on some of the Southern shores, usually alone on sandy areas in calm lagoons near seagrasses. Elsewhere, they are found on intertidal shores with coarse sand, 3 – 4 cm in diameter. The shell is circular with a straight portion on one side. With thick 'ribs' made of up large squarish beads. Usually the color is plain white without any patterns, although there may be irregular blotches which seem to be discoloration or algae growth. *G. tumidum* is more 'swollen' or rounded with rougher ribs. Similar to *G. divaricatum*, it can be distinguished by the more pronounced nodules at the confluence of the posterior and central areas of the flank and distinguishable by its flatter posterior margin and more inflated valves (Tan et al 2010; Tan & Chou 2000; Abbott 1991). In this respect, the study aims to determine developmental stability via fluctuating asymmetry of the bivalve species *G. tumidum* obtained from different environments in Camiguin Island. FA was also used as an indicator of stress. Thus, results of the study would imply that individual FA can be used as a measure of genetic and/or environmental stress and at the same time provide useful information on the nature and variation of *G. tumidum*. Specifically, it aims to investigate differences in the fluctuating asymmetry (right and left valves) of the two populations.

Materials and Methods. The study covered only two areas in Binuni, Camiguin Island namely: Lagoon and Maak. *G. tumidum* were collected from these two sampling stations. Camiguin Island, is a small island far north of Iligan City, a part of Mindanao. It is less industrialized and serves as a tourist spot. The island is separated from the main land by Macalajar Bay in the south and Butuan Bay in the east. The island is totally of volcanic origin, with an active Mt. Hibok-Hibok, a volcano which is 5,246 feet high. A total of 99 samples of *G. tumidum* were collected in which one population with 33 samples came from Lagoon (9° 07' 30"N, 124°47' 15.2"E) and two populations (34 and 32 samples) in Maak (9° O7' 10.39"N , 124° 47' 08.52"E) [20].

Figure 1. Map of Camiguin Island.

The samples were randomly chosen and were photographed from the abaxial part together with a ruler as a size reference using a digital camera for both left and right sides respectively. Morphometric analysis was based on unambiguous and repeatable anatomical marks of the clam. A total of 13 anatomical and mathematical landmarks were used and digitization was done using the TpsDig program (Table 1 & Figure 2).

Table 1

Position of the thirteen landmarks selected in the interior valve of *Gafrarium tumidum*

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

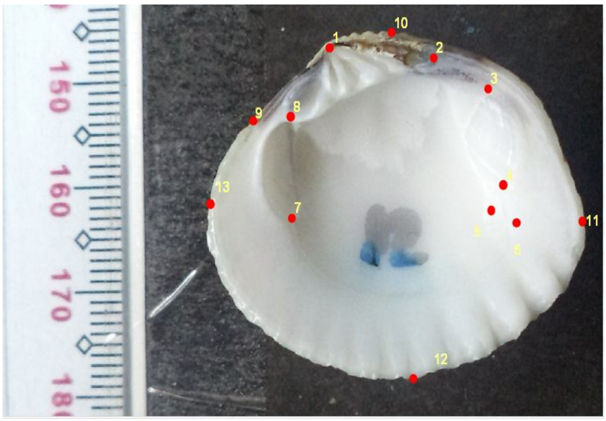

Figure 2. Location of the 13 landmarks on bivalve interior of *Gafrarium tumidum.*

The levels of FA were obtained 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 using a configuration protocol. The projection of homologous landmarks on the tangential space was used taking into account the angle to the zero point. Thus, each point received new coordinates *(XY)* in the tangential space; the set of points was concentrated around the point (00). Antisymmetry was tested on tabulated data of kurtosis. If the value of kurtosis of the difference *(XYr - XYl)* is higher than the tabulated values, it signalled the presence of antisymmetry, i.e. the presence of significant deviation from the normal distribution. Procrustes superimposition analysis was performed with the original and mirrored configurations of the valves simultaneously using the SAGE program. The software analyzed the coordinates of the landmarks per individual, using a configuration protocol for both 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 (Marquez 2006; Klingenberg et al 1998). 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. Sides (directional asymmetry; DA), Individual x sides (fluctuating asymmetry; FA), and their respective error were included as effects. 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. Only Individual x Sides interaction denotes fluctuating asymmetry (FA) (Samuels et al 1991; Palmer & Strobeck 1986, 2003; Carpentero & Tabugo 2014).

To detect the components of variances and deviations, a Procrustes ANOVA was used. Principal Component Analysis (PCAs) of the covariance matrix associated with the component of FA variation were also performed for the samples to carry out an interpolation based on a thin-plate spline and then visualize shape changes as landmark displacement in the deformation grid (Marquez 2006).

Results and Discussion. Fluctuating asymmetry (FA) 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. It is considered to reflect a population’s average state of adaptation and coadaptation (Graham et al 2010; Parsons 1990). Moreover, it is thought to increase under either environmental or genetic stress (Graham et al 2010). An underlying hypothesis of FA analysis is that the development of the two sides of a bilaterally symmetrical organism is often influenced by identical genes and thus, non-directional differences between the sides must be environmental in origin and reflect accidents occurring during development (Palmer 1994; Valen 1962; Gangestad & Thornhill 1999). Fluctuating asymmetry of the right and left valves of *G. tumidum* were assessed through Procrustes method using SAGE software.

Index of FA using the coordinates was determined by including the product of the coordinates of the left and right homologous which provided the final result of the Procrustes ANOVA (Table 2). It was noted that 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. Only Individual x Sides interaction denotes fluctuating asymmetry (FA) (Palmer & Strobeck 1986; Galbo & Tabugo 2014). Hereby, the interaction of ‘*Side x Individuals*’ showed a high value of mean square and a low value of mean square measurement error. Thus, the F value suggested highly significant FA for all samples of *G. tumidum* from two sampling sites of Camiguin Island (Lagoon and Maak) where *$P<0.001$ where, Lagoon exhibited a higher FA value. The results of the Procrustes ANOVA indicated a random variation (FA) between the left and the right sides of the landmark parts of the bivalve shell in two different sites of Camiguin Island, rather than non-random differences among sides.

In this respect, stress in this field can be clearly manifested as high levels of asymmetry. A possible explanation for high levels of FA arises from the differences in genetic composition of the populations resulting in different tolerance to stress. Individuals in their respective locations might have experienced developmental perturbations/noise early in life which resulted to the observed deviations from bilateral symmetry based on the trait examined. Possible sources of developmental noise include exogenous and endogenous stresses such as low habitat quality to low genetic heterozygosity among others (Utayopas 2001). Moreover, according to Mpho et al (2000), the possile causes of developmental instability were well studied and include a wide range of environmental factors (e.g. deviant climatic conditions, food deficiency, parasitism, pesticides) and genetic factors (e.g. inbreeding, hybridization, novel mutants). Such factors may also increase stress to populations. Thus, FA can be used as an indicator of individual quality and adaptation thereby, also demonstrating the potential for FA as a biomarker of stress and developmental instability of populations.

Table 2

Procrustes ANOVA results of *Gafrarium tumidum* from Lagoon and Maak, Camigiun

Effects	*SS*	*Df*	*MS*	*F*	*Remarks*
	Lagoon *G. tumidum*				
Sides	0.0009573	22	4.35E-05	0.21493	-
Individual x sides	0.14252	704	0.00020245	1.8627	highly significant
Measurement error	0.15781	1452	0.00010868	-	not significant
	Maak *G. tumidum*				
Sides	0.0026004	22	0.0001182	0.51331	-
Individual x sides	0.33435	1452	0.00023027	1.7225	highly significant
Measurement error	0.3941	2948	0.00013368	-	not significant

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.

Landmark data allow the differences between population means, or the deviation an individual from its population mean to be visualized in at least two ways. Another way to examine the variability of landmark points in tangent space is to run a principal component analysis (PCA) on the tangent coordinates derived from Procrustes analysis. First principal component depicts vectors at landmarks that show the magnitude and direction in which that landmark is displaced relative to the others. The second depicts the difference via the thin plate splines, an interpolation function that models change between landmarks from the data of changes in coordinates of landmarks. Here, 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 (Marquez 2006).

Based on the percentage of overall variation exhibited by PC1 and PC2, the population from Lagoon exhibited more variation compared to those from Maak. Thus, higher FA was also exhibited by samples coming from Lagoon where, PC 1 accounts for most of the variation (Table 3 & Figure 3).

Table 3

Variance explained by first two principal components between populations of *Gafrarium tumidum* from Lagoon and Maak, Camigiun

G. tumidum	*PC 1 (%)*	*PC 2 (%)*	*Overall (%)*
Lagoon	25.84	21.87	47.71
Maak	22.41	18.60	41.07

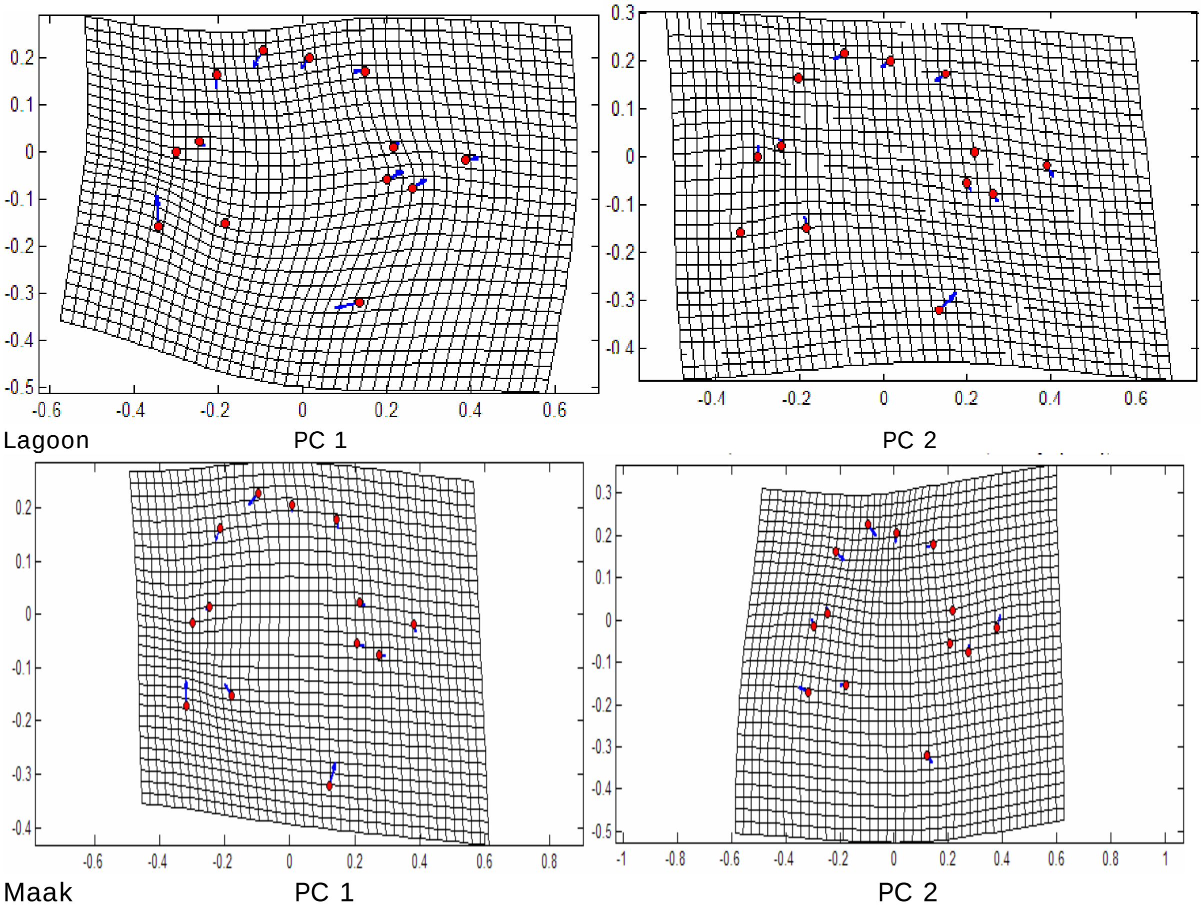

Figure 3. PCA implied deformation for individual x side interaction of fluctuating asymmetry of *Gafrarium tumidum* in Lagoon and Maak.

Conclusions. This study demonstrates the potential of FA as a biomarker of stress and its efficacy in measuring developmental instability in *G. tumidum.* Results yield that species from Lagoon have higher FA than species from Maak. This could be due to differences in the degree of disturbances present in the area, though there is little to noted variation on the morphological aspect. Thus, suggesting that fluctuating asymmetry increases in coastal areas. The results of the study implies that individual FA can be used as a measure of genetic and/or environmental stress and at the same time provide useful information on the nature and variation of *G. tumidum* in Camiguin Island. Increase levels of FA have possible implications on species fitness and adaptation. Knowledge of the various species and their population dynamics is essential in developing programs to preserve the biological diversity of coastal ecosystems.

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References

Abbott R. T., 1991 Seashells of South East Asia. Graham Brash, Singapore, pp. 145.

Bjorksten T., Pomiankowski A., Fowler K., 2001 Temperature shock during development fails to increase the fluctuating asymmetry of a sexual trait in stalk-eyed flies. Proc Biol Sci 268(1475):1503–1510.

Carpentero E. R., Tabugo S. R. M., 2014 Determining Developmental Instability via Fluctuating Asymmetry in the shell shape of *Arctica islándica* Linn. 1767 (ocean quahog). European Journal of Zoological Research 3(3):1-7.

Clarke G. M., 1998 The genetic basis of developmental stability: IV. Individual and population asymmetry parameters. Heredity 80:55–561.
David P., Hingle A., Greig D., Rutherford A., Pomiankowski A., Fowler K., 1998 Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proc R Soc London B Biol Sci 265:1–6.
Galbo K. R., Tabugo S. R. M., 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.
Gangestad S. W., Thornhill R., 1999 Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. J Evol Biol 12:402–416.
Graham J. H., Raz S., Hagit H., Nevo E., 2010 Fluctuating Asymmetry: methods, theory and applications. Symmetry 2:466-495.
Graham J. H., Freeman D. C., Emlen J. M., 1993 Developmental stability: A sensitive indicator of populations under stress. In: Environmental toxicology and risk Assessment, ASTM STP 1179. Landis W. G., Hughes J. S., Lewis M. A. (eds), American Society for Testing Materials, Philadelphia, PA.
Klingenberg C. P., McIntyre G. S., Zaklan S. D., 1998 Left-right asymmetry of fly wings and the evolution of body axes. . Proc R Soc London B Biol Sci 265:1255–1259.
Leong R., de la Seña C. A., Torres M. A. J., Demayo C. G, 2013 Describing morphological and enzyme polymorphism in the Ribbed Venus Clam *Gafrarium tumidum* from five marine coastal locations in Mindanao, Philippines. AACL Bioflux 6(3):1-13.
Marquez E., 2006 Sage: symmetry and asymmetry in geometric data. Ver 1.0. http://www.personal.umich.edu/~emarquez/morph/
Mpho M., Holloway G. J., Callaghan A., 2000 The effect of larval density on life history and wing asymmetry in the mosquito *Culex pipiens.* Bull Entomol Res 90:279-283.
Palmer A. R., Strobeck C., 2003 Fluctuating asymmetry analyses revisited. In: Developmental Instability: causes and consequences. Polak M. (ed), Oxford University Press, New York, USA.
Palmer A. R., Strobeck C., 1986 Fluctuating asymmetry - measurement, analysis, patterns. Annu Rev Ecol Evool Syst 17:391-421.
Palmer A. R., 1994 Fluctuating asymmetry analysis: a primer. In: Developmental Instability: its origins and evolutionary implications. Markow T. A. (ed), Kluwer Academic, London.
Parsons P. A., 1961 Fly size, emergence time and sternopleural chaeta number in Drosophila. Heredity 16:455–47.
Parsons P. A., 1962 Maternal age and developmental variability. J Exp Biol 39:251–260.
Parsons P. A., 1990 Fluctuating asymmetry: an epigenetic measure of stress. Biol Rev Camb Philos Soc 65(2):131–145.
Parsons P. A., 1992 Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity 68:361–364.
Pomiankowski A., 1997 Genetic variation in fluctuating asymmetry. J Evol Biol 10:51–55.
Rasmuson M., 2002 Fluctuating asymmetry indicator of what? Hereditas 136:177–183.
Roy B. A., Stanton M. L., 1999 Asymmetry of wild mustard, *Sinapis arvensis* (Brassicaceae), in response to severe physiological stresses. J Evol Biol 12:440–449.
Samuels M. L., Casella G., McCabe G. P., 1991 Interpreting blocks and random factors: rejoiner. J Am Stat Assoc 86:798-808.
Tan K. S., Henrietta P., Woo M., 2010 Preliminary checklist of the molluscs of Singapore (pdf), Raffles Museum of Biodiversity Research, National University of Singapore.
Tan K. S., Chou L. M., 2000 A guide to the common seashells of Singapore. Singapore Science Centre, pp. 160.
Utayopas P., 2001 Fluctuating Asymmetry in fishes inhabiting polluted and unpolluted bodies of water in Thailand. Thammasat Int J Sc Tech 6(2):10-20.
Valen V., 1962 A Study of fluctuating asymmetry. Evolution 16:125-142.
Van Dongen S., Lens L., 2000 The evolutionary potential of developmental instability. J Evol Biol 13:326–335.

Van Velen L., 1962 A study of fluctuating asymmetry. Evolution 16:1-7.
Velickovic M., 2004 Chromosomal aberrancy and the level offluctuating asymmetry in black-striped mouse (*Apodemus agrarius*): effects of disturbed environment. Hereditas 140:112–122.
Vollestad L. A., Hindark, Moller A. P., 1999 A meta-analysis of fluctuating asymmetry in relation to heterozygosity. Heredity 83:206–218.
Waddington C. H., 1942 Canalization of development and the inheritance of acquired characters. Nature 150:563-565.
Zakharov V. M., 1989 Future prospects for population phenogenetics. Soviet Scientific Reviews Series, Section F., Routledge.

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