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Enzyme polymorphism among natural populations of damselfish, *Stegastes nigricans* cuvier collected in selected reefs of Iligan Bay and Camiguin island, Philippines

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ABSTRACT

Variability in esterase (EST), alkaline phosphatase (AlKp) glutamate oxaloacetate transaminase (GOT) and aspartate aminotransferase (AAT) loci in the plasma (pl), epaxial muscle (mus) and liver (li) tissues within and among the local populations of damselfish, Stegastes nigricans, was conducted using horizontal starch gel electrophoresis. Samples were obtained from four selected reef sites along Iligan Bay; Clarin (Misamis Occidental), Buru-un (Iligan City), Lugait (Misamis Oriental) and in Sagay (Camiguin Islands). Allelic frequencies in each protein locus were computed as basis in comparing variations within and among populations. Results revealed that values on the number of effective alleles (n_e), heterozygosity values and allelic differentiation (δ_T) of the 4 gene loci showed that Buruun (2.142, 0.546) and Lugait (2.111, 0.540) are more similar than Clarin (1.880, 0.468) and Sagay (1.8166, 0.449). These results suggest that expression of genetic variability among natural populations could be possibly due to environmental heterogeneity with stable and unstable habitats.

Keywords: S. *nigricans*, genetic variability, polymorphism, environmental heterogeneity

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INTRODUCTION

Variability is the fundamental and basic characteristic of life. Every level of organization of life displays variation in some parameters, in space or time, within and between cells, tissues, organisms, populations and communities. The existence of variations in natural populations of organisms is a necessary condition for evolution. While variability is both a product and foundation of the evolutionary process, biologists are still confronted with the basic problems as to how to explain the nature, extent and causes of the overwhelming complexity. Morphological differentiation is one of the several approaches which proved useful in studying variability. Morphological data alone, however, is insufficient to explain variability. Molecular biology, biochemistry and other tools coupled with morphology are powerful tools in understanding variability and evolutionary relationships among and within populations of organisms [1-10].

It was therefore the major objective of the study to investigate genetic variation in one of the organisms found in found around coral reefs at a depth of 1 to 12 meters, the damselfish *Stegastes nigricans* or dusky farmerfish (Fig. 1). This species practises a form of agriculture with a species of red algae. The fish will claim a patch of "brown carpet algae" which it defends by chasing away other fish and sea urchins. The fish also pulls up other bits of algae that attempt to grow in the patch and swims outside of its territory to spit the invading algae out. When the fish claiming a patch is removed, the patch is eaten up within a few days. When a patch of the brown carpet algae is caged to keep both *S. nigricans* and other fish out of the patch, other species of algae quickly overwhelm the patch. This seems to indicate that the brown carpet algae is dependent on *S. nigricans* for its survival [11]. In a study on a disturbed and an undisturbed reef, it was observed that the size of the fish as as well as the amounts of utilized

higher organic matter, organic carbon, nitrogen and organic nutrient [12]. The differences observed may indicate genetic diversity between populations of the fish in these two environments of the reef. To determine whether genetic diversity occurs between reef populations of the fish, this study was conducted. Populations of many species of organisms may respond both morphologically and genetically different to a changed environment. Individuals tend to express different phenotypes (morphological, physiological or behavioral) when thriving in varied environment [13]. One sensitive way to assess such genetic variability is through isozyme electrophoretic analysis. Variation in the banding pattern can be directly equated to variation in a gene coding for the variant proteins being studied [14]. Some species of organisms may be suitably used as marker to assess the adaptive value of electrophoretically-detectable proteins. It could also be used to support variability among natural populations of the same species as a result of environmental heterogeneity.

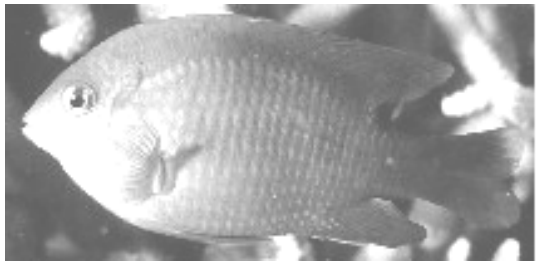

Figure 1. Adult *Stegastes nigricans*

MATERIALS AND METHODS

Forty live sexually mature specimens of damselfish, *Stegastes nigricans*, were collected from the coastal waters along Iligan Bay; Clarin (Misamis Occidental), Buru-un (Iligan City), Lugait (Misamis Oriental) and in Sagay (Camiguin Islands) (Fig. 2). Blood was withdrawn directly from the heart of each fish and placed in an eppendorf tube containing 5 % sodium citrate (0.5 ml). Blood samples were immediately spun at 5000 revolutions per minute (rpm) for 5 minutes at 4^{o} C to fractionate the plasma from the erythrocytes. The plasma were transferred to clean eppendorf tubes and were kept frozen until analysis.

Figure 2. Map of the sampling areas (Clarin, Mis. Occ., Buruun, Iligan City, Lugait. Mis. Or. Sagay, Camiguin)

Epaxial muscles and liver of each fish were removed, washed with distilled water and homogenized in a glass homogenizer with 1 % glycerine. The homogenates were placed in clean eppendorf tubes and were spinned at 6000 rpm for 6 min at 4^{o} C. The supernatants were pipetted to clean eppendorf tubes and were kept frozen until analysis.
Electrophoretic analyses of the different proteins, namely; plasma, epaxial muscles and liver esterase (*EST*), alkaline phosphatase (AlKp), glutamate-oxaloacetate transaminase (GOT) and aspartate aminotransferase (AAT)

were done using horizontal electrophoresis system, Model OSP 135. Twelve percent starch solution in Tris-Citrate gel buffer (pH 8.6) and Borate electrode buffer (pH 8.0) was used for both plasma and liver *EST* and AlKp. Activity of the esterase was determined in an assay solution containing Fast Blue RR in 0.5 Tris-HCl (ph 7.1) and α- and β-naphthol acetates as subtrates. For alkaline phosphatase, assay solution containing Fast Blue RR in $MgSO_4$.d H_2O and β-naphthyl Na phosphate as substrates was used [15].

Identification and nomenclature of the protein-coding locus was patterned after the protocol of Shaklee et al. [16]. Genetic variability of the gene loci was determined based on 1% criterion of polymorphism ($0.01<q<0.99$). The effective number of alleles (n_e) and total population differentiation (δ_T) were calculated using the following formulae:

$n_e = 1/(\sum X_i^2)$ where X_i =allelic frequency at a given locus and total effective number of alleles is equal to the geometric mean of all n_e's.

$\delta_T = [N/N-1]\ [1-\sum X_i^2]$, where N equals to population size and x_i is the relative allelic frequencies. The unweighted pair-group arithmetic average clustering method (UPGMA) was used in the construction of dendrogram [14].

RESULTS AND DISCUSSION

Polymorhism was observed in the four presumptive enzyme loci in *S. nigricans* within and among natural populations sampled from Iligan Bay and Camiguin Islands. Almost all natural populations of *S. nigricans* showed polymorphic expression except for pl AlKp in Clarin and Sagay populations. Table 2 summarizes the allelic frequencies of alleles in *pl*, *mus* and *li* of *S. nigricans*. In plasma and liver EST locus, polymorphism is governed by three codominant alleles in all sites except for Sagay liver EST (Camiguin Islands) whereas for li AlKp, GOT (mus and li) and AAT (mus and li) loci are governed by two segregating alleles. The *pl* AlKp loci in Clarin and Sagay are monomorphic.

Table 1. Polymorphism in the Esterase (EST), Alkaline Phosphatase (AlKp), Glutamate-oxaloacetate transaminase (GOT) and Aspartate aminotransferase (AAT) in Plasma (pl), Epaxial muscle (mus) and Liver (li) tissues of *S. nigricans* from Four Selected Sites (Clarin, Buru-un, Lugait and Sagay)

Gene locus	EST		AlKp		GOT		AAT	
Site/Tissue	**pl**	**li**	**pl**	**li**	**mus**	**li**	**mus**	**li**
Clarin	P	P	m	P	P	p	p	P
Buruun	P	P	P	P	P	p	p	P
Lugait	P	P	P	P	P	p	p	P
Sagay	P	P	m	P	P	p	p	P

P – polymorphic, m – monomorphic

Table 2. Allelic frequencies in each protein locus (EST, AlkP, GOT and AAT) of plasma (pl), epaxial muscle (mus) and liver (li) tissues of *S. nigricans* from Lugait, Buruun, Clarin and Sagay

LOCI		Allele	CLARIN	BURUUN	LUGAIT	SAGAY
EST	*pl*	112	0.499	0.403	0.387	0.411
		113	0.483	0.338	0.372	0.518
		116	0.172	0.258	0.242	0.071
	li	113	0.37	0.317	0.233	0.411
		115	0.481	0.399	0.363	0.589
		117	0.148	0.383	0.383	0
Alkp	*pl*	108	1.0	0.617	0.594	1.0
		110	0	0.383	0.406	0
	li	104	0.50	0.536	0.625	0.562
GOT	*mus*	102	0.541	0.593	0.6	0.312
		105	0.452	0.406	0.4	0.688
	li	104	0.469	0.577	0.672	0.533
		107	0.531	0.423	0.328	0.467
AAT	*mus*	102	0.469	0.469	0.387	0.437
		105	0.531	0.531	0.613	0.563
	Li	104	0.352	0.345	0.607	0.484
		107	0.648	0.655	0.393	0.516

Table 3 presents values on the total effective number of alleles (n_e) and total population differentiation (δ_T). These two values are measures of genetic variation within and among populations. The table shows that Clarin (n_e =1.880; $\delta_{T\%}$ = 46.82) and Sagay (n_e = 1.8166; $\delta_{T\%}$ = 44.86) are more similar compared to Lugait (n_e = 2.111; $\delta_{T\%}$ = 54.04) and Buruun (n_e =2.142 $\delta_{T\%}$ = 54.61). The number of effective alleles is correlated with the total population

differentiation. Genetic heterogeneity offers greater species flexibility and adaptability to environmental changes[14].The mean value indicate that Lugait and Buruun populations are than those in Clarin and Sagay.

Table 3. Effective number of alleles (n_e) and total population differentiation (δ_T) in each protein locus (EST, AlKp, GOT and AA7) of plasma (pl) and liver (li) of *S. nigricans* from Clarin, Buru-un, Lugait and Sagay

		Clarin		Buruun		Lugait		Sagay	
		n_e	δ_T	n_e	δ_T	n_e	δ_T	n_e	δ_T
EST	Plasma	2.07	0.537	2.91	0.68	2.88	0.68	2.27	0.58
	Liver	2.56	0.63	2.93	0.68	3.0	0.69	1.94	0.50
Alkp	plasma	1.0	0	1.89	0.49	1.93	0.50	1.0	0
	Liver	2.0	0.52	1.99	0.51	1.88	0.48	1.97	0.51
GOT	Muscle	2.02	0.52	1.93	0.51	1.92	0.51	1.97	0.53
	Liver	1.99	0.51	1.95	0.51	1.19	0.47	1.92	0.50
AAT	Muscle	1.99	0.53	1.99	0.52	1.91	0.50	1.97	0.53
	Liver	1.84	0.47	1.83	0.47	1.91	0.495	2.0	0.52
Total n_e	1.880		2.142		2.111		1.8166		
He	0.5522		0.47799		0.5155		0.5684		
Mean δ_T		0.4682		0.5461		0.5404		0.4486	

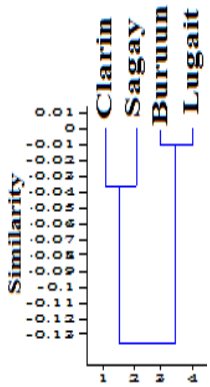

Figure 3. Dendrogram of *S. nigricans* from four sampling sites, Lugait (1), Buruun (2), Clarin (3) and Sagay (4) based on UPGMA genetic identity

There are several possible explanations of the results in this study. The damselfish being highly territorial and non-migratory is always affected by its habitat which is frequently the depository for organic pollutants, agricultural and industrial wastes [17]. Studies of disturbed and undisturbed reefs suggested that eutrophication is the main disturbance factor accounting for the recorded differences[12]. Other studies conducted on large and majority island populations of this species was reported to have less allozyme genetic variation than their mainland counterparts [18]. Likewise, a related study showed genetic profiles observed in *Stegastes* populations indicate a higher genetic variability along the shoreline than at oceanic sites that is related to a reduced effective population size on islands [19]. This could be one of the possible reasons also for the variations in *S. nigricans* populations thriving in Iligan Bay and Camiguin Is.

Genetic responses of populations, both in cages and in natural environments, to ecological variables may also explain the results generated. A study had demonstrated that variation at three of twelve polymorphic isoenzyme loci from the warm water population of a marine fish, *Fundulus heteroclitus,* was beyond the range of variation among control populations, and resembled those determined for populations living at more southern latitudes [20]. Such differences were interpreted as adaptation to warm environments. Greater variations of gene loci expression in coral reef fishes in Lugait and Buruun maybe related to greater environmental disturbance in those sites.

While no analysis was made on the different reefs from many different locations in the current study, it can be argued that the variations observed can be associated with environmental disturbances and with other fitness-related traits [21]. Allozyme polymorphism is an adaptive strategy among many organisms as it provides metabolic plasticity in response to long term environmental changes [22].

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