Genetic Variability in Populations of the Giant Toad *Bufo marinus* Linn.

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Abstract

Genetic variability in 289 Philippine giant toads **Bufo marinus** *Linn. from three populations was assayed by starch gel electrophoresis of 8 blood proteins encoded by 12 presumptive loci. Various indices of intrapopulation genetic variation showed the Mindanao toad population to be most variable (P=75%; H=32.25% and A=2.167) followed by the Luzon group (P=66.67%; H=16.27% and A=2.167). The third population, from the Visayas, showed the least variability (P=16.67%; H=8.26% and A=1.5). Estimates of Nei's and Hillis' genetic identity and distance and Hedrick's genotypic identity showed that the Luzon and Mindanao populations share many alleles in common (I=0.9514, I^k=0.9505 and I_H=0.8963) and are therefore more closely related. Consequently, greatest genetic divergence exists between the Luzon and Visayas populations.*

Keywords: *Bufo Marinus,* blood proteins, polymorphism, electrophoresis

Introduction

The giant toad *Bufo marinus* Linn. occupies a wide range of habitats in many islands of the Philippines. A native of South America, this species was introduced by humans to many places of the world especially the Pacific Islands including the Hawaiian islands, the Marianas, Solomons and the Admiralty islands (Rabor, 1981) and to other sugar-producing countries of the world to control insect populations mainly beetles in the sugar fields (Alcala, 1986). In 1934, the toad was introduced to the Philippines (Merino, 1936).

In the laboratory, the toad has been a favorite biological material for simple routine anatomical dissections and in physiological experiments in the muscles and nerves. Adult males are used in routine pregnancy tests. The potential of the toad as fish meal substitute in poultry diets (Basuel, 1983) and as a biological indicator (Amarillo, 1992) is promising. Being highly adaptable to various habitats, the toad may be regarded as a `canary in the coal mine' that bespeaks of the changes in the environment. The archipelagic nature of the country provides geographic barriers which restricted interisland migration of *B. marinus* and allowed continuous interbreeding within the populations. This makes the populations of *B. marinus* excellent subjects for genetic differentiation and evolutionary studies.

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This paper attempts to investigate the genetic variability in three populations of giant toad *Bufo marinus* in the Philippines based on electrophoretic analysis of 8 blood proteins.

Materials and Methods

Two-hundred and eighty-nine adult *B. marinus* were collected from three sampling sites in the country, namely: 101 from Luzon area (Los Banos and Pansol, Calamba), 95 from the Visayas area (Catarman, Northern Samar) and 93 from the Mindanao area (Iligan City and Plaridel, Misamis Occidental). Whole blood was drawn by ventricular puncture and was placed in a centrifuge tube containing an anticoagulant (5 percent sodium citrate). Uncoagulated blood samples were fractionated by centrifugation at 1500 revolutions per minute (rpm). Plasma and erythrocytes were stored at -20°C.

Electrophoretic analysis of the blood proteins was performed at the Isozyme Laboratory of the International Rice Research Institute (IRRI) using Tris-Citrate (pH 8.8) as the gel buffer and Sodium-Borate (pH 8.2) as electrode buffer (Glaszmann et al., 1990) using 14% starch (Sigma) concentration. Samples along with the check sample and standard albumin and transferrin were loaded on the slit made in one end of the gel using filter paper wicks (Whatmann no. 3). Bromphenol blue was used as tracking dye. A potential difference was applied through the gel. At the initial voltage of about 10 volts/cm, electrophoresis was stopped after 4 hours. Assay of enzyme activity and staining were done following Parker (1971) and Shaw and Prasad (1970).

Gene nomenclature follows Shaklee et al. (1990). Gene and genotypic frequencies were estimated based on the bands that were obtained from each locus. Within population variability was assessed using three indices, namely: proportion of polymorphic loci *(P)* based on the 95% criterion of polymorphism, average heterozygosity in the population *(H)* according to Nei (1975), and number of alleles per locus *(A)*. Interpopulation variability was assessed based on Nei's genetic identity and distance (Nei, 1972), Nei's modified genetic identity and distance (Hillis, 1984) and Hedrick's proportion of genotypic identity (Hedrick, 1971).

Results and Discussion

Albumin (ALB). Albumin showed the fastest mobility among the anodally-migrating proteins. Two ALB phenotypes were observed in toad samples from the Luzon and Mindanao areas while only one phenotype was observed from the Visayas samples. However, following the 95% criterion of allelic polymorphism, only the Mindanao population exhibited polymorphism at this locus. The observed banding pattern in the ALB phenotypes gave the impression that the locus is governed by two autosomal codominant alleles.

Transferrin (TF). Only one phenotype of TF was observed in all the toad samples collected from three areas.

Other plasma proteins. Three unidentified proteins (designated as PROT1, PROT2 and PROT3) were observed behind the transferrin plate. The Luzon and Mindanao populations showed three phenotypes for PROT1 and were found to exhibit polymorphism at this locus. All three populations were found to be polymorphic at the *PROT2* locus, as two phenotypes were resolved indicating this locus is governed by at two codominant alleles. All three toad populations were monomophic in the *PROT3* locus.

Erythrocyte Esterases (EST). At least 5 presumptive *EST* loci (designated *EST* 1 to 4 *EST*) were observed in the Luzon and Mindanao toad populations and at least loci (designated *EST* 1 to 7) were found in toads from the Visayas area using ∝-naphthyl acetate and β-naphthyl acetate as substrates. This is based on the common observation that some faint, although not clearly resolved, bands were still visible in the more anodal region of the gel. In all populations, the EST bands differed in their staining intensity, more intense bands were found near the origin becoming faint as the proteins moved further to the anodal end. In both Luzon and Mindanao populations, *EST* 1 to 4 migrated anodally while *EST* 7 migrated towards the cathode. At least three phenotypes each for *EST* 1 to 4 were observed, and all two populations were found to exhibit polymorphism in all 5 presumptive loci. The banding patterns observed in *EST* 1 to 4 phenotypes suggested that each *EST* locus is controlled by three autosomal codominant alleles. EST 7, on the other hand, showed three phenotypes indicating that the *EST*-7 locus is governed by two autosomal codominant alleles. Of the esterases in samples from the Visayas area, 6 migrated anodally while one migrated towards the cathode. Only one electromorph for each of *EST* 2 to 5 was observed indicating that the loci were fixed at a certain allele. *EST* 6 and 7 showed two and three phenotypes, respectively, but absence of polymorphism in these two *EST* loci was observed because the allelic frequencies did not satisfy the 95% criterion.

Malate Dehydrogenase (MDH). Three distinct bands and one faint band closest to the origin can be readily observed, all migrating anodally. It has been reported that in many species of fish and in most amphibians, the sMDHs appear as 3 sharp, equally spaced anodal bands, each band being a dimer (Bailey et al., 1969; 1970; Schwantes and Schwantes, 1977; 1982). Furthermore, the sMDHs were shown to migrate more anodally during electrophoresis than the mMDHs in amphibians, reptiles and mammals. Since the RBC hemolysate in the current work was not subjected to fractionation. the observed MDH electromorphs may be composed of both sMDH and mMDH. The first 3 sharp anodal bands most likely make up sMDHs while the faint band nearest the origin make up the mMDH. As such, it appears that all the toad samples were heterozygotes for the sMDH isoloci. The mMDH, on the other hand, seems to be fixated at one allele.

Hemoglobin (HB). Only one band of HB was observed migrating anodally for three populations.

Intrapopulational Variability

Tables 1 to 3 present the allelic frequencies of the 12 presumptive loci encoding the 8 blood proteins as resolved by starch gel electrophoresis. Based on these data the various estimates of within population variability in the toad populations in three sampling areas were calculated (Table 4). The mean average heterozygosity for the three populations is 18.93% with the Mindanao having the highest value (32.25%) and Visayas the least value (8.26%). Likewise, the Mindanao together with the Luzon toad populations had the same average number of allele per locus (2.167) higher than those in the Visayas. The average proportion of polymorphic loci in all three populations was 52.78%, the highest value was observed in Mindanao population (75%) while the lowest value was in the Visayas (16.67%). These observations are comparable to those reported in some other species of *Bufo* (Rogers, 1973; Guttman, 1975; Matthews, 1975).

Table 1. Frequencies of alleles at 12 presumptive loci in the population of toad *B. marinus* Linn. from Luzon area.

LOCUS	ALLELE FREQUENCY		
	*1	*2	*3
ALB	1.0000		
TF	1.0000		
PROT-1	0.0119	0.9464	0.0416
PROT-2	0.1099	0.8406	0.0495
PROT-3	1.0000		
EST-1	0.0489	0.9239	0.0271
EST-2	0.0341	0.8977	0.0612
EST-3	0.0476	0.8750	0.0774
EST-4	0.0506	0.8987	0.0506
EST-7	0.7957	0.2043	
HB	1.0000		
MDH	0.5000	0.5000	

Table 2. Frequencies of alleles at 14 presumptive loci in the population of toad *B. marinus* Linn. from Visayas area.

LOCUS	ALLELE FREQUENCY		
	*1	*2	*3
ALB	0.9628	0.0372	
TF	1.0000		
PROT-1	1.0000		
PROT-2	0.2808	0.7192	
PROT-3	1.0000		
EST-1	0.0006	0.9775	0.0168
EST-2	1.0000		
EST-3	1.0000		
EST-4	1.0000		
EST-5	1.0000		
EST-6	1.0000		
EST-7	0.9943	0.0006	
HB	1.0000		
MDH	0.5000	0.5000	

Table 3. Frequencies of alleles at 12 presumptive loci in the population of toad *B. marinus* Linn. from Mindanao area.

LOCUS	ALLELE FREQUENCY		
	*1	*2	*3
ALB	0.8613	0.1386	
TF	1.0000		
PROT-1	0.0462	0.8721	0.0462
PROT-2	0.2778	0.7222	
PROT-3	1.0000		
EST-1	0.2128	0.5904	0.1915
EST-2	0.3387	0.5860	0.0752
EST-3	0.1887	0.7142	0.0969
EST-4	0.5423	0.4406	0.0169
EST-7	0.9032	0.0968	
HB	1.0000		
MDH	0.5000	0.5000	

Table 4. Estimates of intrapopulation variability in three populations of toad *B. marinus* Linn.

PARAMETER	POPULATION		
	Luzon	Visayas	Mindanao
1. Average heterozygosity (*H*)	16.27%	8.26%	32.25%
2. Average number of alleles/locus (*A*)	2.167	1.5	2.167
3. Proportion of polymorphic loci (*P*)	66.67%	16.67%	75.00%

Table 5. Genetic identity and distance values based on the 12 presumptive loci among the three populations of toad *B. marinus* Linn.

	LUZON	VISAYAS	MINDANAO
LUZON	—	0.9046 (0.6747)	0.9514 (0.9505)
VISAYAS	0.1002 (0.3935)	–	0.8557 (0.7884)
MINDANAO	0.0498 (0.0508)	0.1559 (0.2372)	—

Values above the diagonal represent genetic identity; Nei's I without parenthesis and Hillis' I enclosed in parenthesis.*

Values below the diagonal represent genetic distance; Nei's D without parenthesis and Hillis' D enclosed in parenthesis.*

Interpopulation Variability

Table 5 shows the genetic identity and distance values between populations based on the 12 presumptive loci surveyed. Both Nei's (1972) and Nei's modified (Hillis, 1984) estimates of genetic identity showed the Mindanao and Luzon populations having the highest degree of relatedness and therefore sharing the most number of alleles in common (I=0.9514 and I*=0.9505). On the other hand, the Luzon and Visayas populations shared the least number of alleles in common thereby showing the lowest degree of relatedness. Hedrick's (1971) proportion of genotypic similarity confirms this observation where the Mindanao and Luzon populations showed the highest value (I_H =0.8963). Between Visayas and Mindanao populations, I_H=0.7964 while lowest similarity was found between Luzon and Visayas populations (I_H=0.7553). As the three populations showed varying degree of genetic closeness, it therefore follows that the extent of genetic divergence between the populations varied. The smallest genetic distance was observed between the Luzon and Mindanao toad populations (D=0.0498 and D*=0.0508) while there may be a larger genetic divergence between the Luzon and Visayas toad populations.

General Discussion

The wide range of *H* and *P* values and the difference in the *A* values observed in the present investigation only shows that some forces may influence the activity and hence adaptibility of certain alleles in certain locus. The electrophoretic variability in populations may be influenced largely by both the individual variability at one locus resulting from genetic segregation (Kaminski et al., 1987) and interlocus variation that may have been induced by gene substitution, mutation rate and natural selection among loci (Nei, 1975). This is so because the natural populations include a mixture of loci which are at various stages of evolution.

There is a wide range of variations among proteins, some show a high degree of gene diversity (i.e. esterases) while others show a low gene diversity (i.e. general proteins) (Nei, 1975). The high degrees of polymorphic variability that occur may be attributed to any of a variety of physiological interactions. That polymorphism especially in many enzymes increases the fitness among the individuals by providing means of metabolic plasticity for the varying environment is highly plausible (Johnson, 1974). Soule (1976) proposed a model setting three conditions that must be met before a population can have very high levels of heterozygosity. The population or lineage must be large in order to maintain high levels of polymorphism; old since the new polymorphism seem to appear at a very slow rate; and the population must be evolving quite slowly because directional selection probably erodes heterozygosity.

One puzzling case in point in the present investigation is the absence of polymorphism in all esterases (*EST*) loci in the Visayas population. It has been shown that the functional characteristics of esterases demonstrate a somewhat predictable situation. For instance, in the freshwater fish, *Catostomus clarkii*, the frequency of alleles for esterase shows clinal distribution (Koehn and Rasmussen, 1967; Koehn, 1969). Geographical clinal variation at 7 esterase-coding loci has also been observed in *Zaprionus indianus* (Parkash and Yadav, 1993). In another case, the phenotypic difference in esterases observed in the marine snail, *Littorina littorea*, was thought to reflect difference in environment selection pressure (Matteo, 1975). One known force which leads to a decrease in genetic variability especially in small populations is genetic drift. However, it is strongly believed that all toad populations thoughout the country may have started with relatively few individuals as founders and hence, each population must be presumed to have been subjected to random drift. Whether the observed unusual genic invariability of *B. marinus* in the Visayas area is linked to a peculiar adaptive strategy in some species of toads, or common to all toads or common to anurans with similar strategies or due to some other explanation (Guttman, 1975) is still to be known.

The patterns of genetic relationship based on genetic identity and similarity values observed in the present study may generally correspond to similarities in geographical, physiographic and climatic conditions (Matthews, 1975). Genetic variability plays the role of an adaptive strategy of populations to cope with temporal or spatial heterogeneity of the environment. Population living in heterogeneous habitats should display large genetic variability, and those living in homogeneous and stable habitat should be genetically depauperate (Apfelbaum et al., 1991). The observed difference in the degree of relatedness can correspond to either the individual variability at one locus resulting from segregation and/or interspecific divergencies, past or in the making (Kaminski et al., 1987).

Summary and Conclusion

Starch gel electrophoresis of 8 blood proteins encoded by 12 presumptive loci allowed assessment of genetic variability among and between the populations of the giant toad *B. marinus*. The proteins analyzed were albumin, transferrin, 3 other plasma proteins, erythrocyte esterases and malate dehydrogenases and hemoglobin.

1. Based on the three indices of intrapopulation variability, *H* ranged from 8.26% to 32.25%, *P* ranged from 16.67% to 75%, and *A* ranged from 1.5 to 2.167 in the three populations. The Mindanao toad population apparently possesses the highest within population genetic variability. On the other hand, least intrapopulation variability was observed in the Visayas population.
2. Among the three toad populations, those from Luzon and Mindanao showed the highest degree of relatedness and hence share many alleles in common as indicated by highest identity (*I* and *I** =.9514, and .9505, respectively) values.
3. Likewise, Hedrick's genotypic similarity index showed that both Luzon and Mindanao toad populations consistently have highest genetic similarity (I_H=.8963) followed by the Visayas and Mindanao toad populations (I_H=.7964). The lowest similarity value (I_H=.7553) was observed in the Luzon and Visayas toad populations.
4. The observed differences in the within population variability and in the degree of genetic relatedness between popilations may be ascribed to some specific differences in the environmental conditions such as substrate characteristics.

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