EFFECT OF INBREEDING ON MORPHOLOGICAL TRAITS OF POPULATIONS OF GIANT FRESH WATER PRAWN, *Macrobrachium rosenbergii*: IMPLICATIONS FOR SELECTIVE BREEDING

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ABSTRACT

Genetic improvement through selective breeding relies on the genetically-controlled phenotypic variability in the character of interest. Therefore, the extent of phenotypic variability in the population to be selected is an important parameter, as it potentially influences the population's response to selection. This study was aimed to investigate the effect of inbreeding on the survivability, growth, and phenotypic variability of giant freshwater prawn (GFP), *Macrobrachium rosenbergii*. Two GFP populations of different inbreeding levels, namely 25% and 0%, respectively, were formed by mating of individual broodstock with known pedigree. Study was conducted for two months starting from newly hatched larvae up to nursery stage (30 day-old post larva). Phenotypic variability profile, expressed in the morphometric mean and coefficient of variation (CV) of twenty one morphometric characters were measured and evaluated. Results showed that in general, the inbred populations had lower values in the mean of all characters (100%), indicating that they suffered from inbreeding depression. Similarly, a lower CV values were observed in sixteen (75%) of the morphometric characters measured, indicating a potential reduced of genetic gain when they are used in selective breeding program. These results suggest the importance of controlling inbreeding level in breeding population that adverse effects resulted resulted from inbreeding can be minimized.

KEYWORDS: inbreeding, phenotypic variability, coefficient of variation

INTRODUCTION

The raw material for genetic improvement through selective breeding is the existence of heritable-genetic variation within the character of interest. In practice, this variation is manifested in the form of phenotypic variation, either in morphometric or meristic traits. Population's phenotypic variability, most commonly expressed as size variation, may result from both exogenous and endogenous sources. The exogenous factors include such circumstances as feed competition, social stress, increased motor activity, and dominance cost (Goldan *et al.*, 1997). The endogenous factors such as differences in strain, populations or family (De March, 1997), and differences in genetic variation may also influence the population's size variation.

Inbreeding, which is mating of closely related individuals, reduces genetic variation of populations. Studies using molecular tools in several fish taxa have documented and confirmed this trend. For the purpose of breeding program, particularly those carried out through conventional approach however,

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information of those effect on quantitative traits is more preferable. There are at least two main reasons for this concern. Firstly, most conventional breeding programs work with those traits. Most traits of commercial interest such as growth, disease resistance, and feed consumption efficiency are quantitative traits. Secondly, due to the nature of relatively small and closed population, breeding population could not avoid from the occurrence of inbreeding.

In preliminary study, Imron (2009) reported the effect of inbreeding on the performance of larval stage of GFP including the rate of larval development, survival rate, and growth of the newly hatched larvae up to post larvae (PL) stage. He found that the performance of the inbred group was lower that of the non-inbred counterparts. However, no information was obtained as to the phenotypic variability within that life stage and beyond. This study was aimed to investigate the effect of inbreeding on GFP's phenotypic variability at the later stage of the life.

MATERIALS AND METHODS

Preparation of Test Populations

Test animal consisted of two populations of GFP that were set to have different levels of inbreeding coefficient. The development of the groups was allowed due to the availability of live biological collections of different GFP germ plasm in the Research Institute fo Fish Breeding (RIFB). The first group, called inbred population, was population of progenies produced by mating of individual broodstocks derived from the same parental pair. Given this relationship, inbreeding level in the first group would be at least 25% (Nakadate *et al.*, 2003). The actual inbreeding level may exceed that figure depending on the previous parental (grandparent) relatedness. However, due to lack of that information, it was assumed that individuals within grandparent generation were not related. The second group, called outbred population, was progenies produced by mating individual broodstocks derived from the same population but of different parental pair (Figure 1). Under these circumstances, the inbreeding level of the second group would be zero.

The two groups of progeny populations differing in inbreeding level, each with four replicates, were reared for two months. Rearing activity was carried out in two stages, namely larval and nursery stages. Larval rearing period started from the newly hatched larvae up to newly metamorphosed-post larvae. Rearing at this stage was conducted in 50 L conical tanks placed inside the hatchery. Subsequent rearing (nursery) was conducted in 1 m x 1 m square hapas installed in outdoor pond. Rearing management including stocking density, feeding practice, and water quality management during larval rearing period across groups was made equal as described by Imron (2009), that comparison among groups was allowable with a minimum bias (Lin & Ritland, 1997).

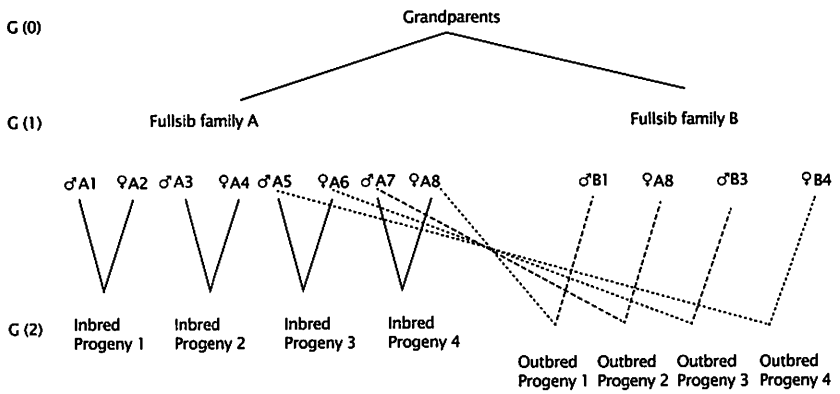

Figure1. Mating design used to produce groups of inbred and outbred progenies. Groups of inbred progenies were produced by brother-sister mating while groups of outbred progenies were produced by mating of individual derived from different families. Population tests were those belong to G (2)

Parameter Examined

The main parameters examined were morphometric traits as presented in Table 1. Measurement was carried out at three day interval using a light microscope equipped with micrometer. Evaluation of phenotypic variability was focused on the measurement of mean and coefficient of variation of twenty two morphometric characters as described in Table 1. Measurement of these characters was conducted at the end of the nursery period by sampling in random thirty individuals per replicate. Growth parameter was assessed using measurement of total length and limited to nursery period. Total 240 individual samples representing the inbred and outbred groups were measured for their phenotypic variability.

Data Analyses

The data of morphometric characters were analysed in two ways, i.e. descriptive-graphical presentation and statistical significance tests. Inbreeding depression coefficient within any characters was assessed using formula $\partial = 1 - X_1/Xo$ (Roff, 1998; Wright *et al.*, 2008); where ∂ was the difference between the inbred and outbred morphometric means and X_1 and Xo represent the mean value of a morphometric character within the inbred and the outbred, respectively. Comparisons of mean of morphometric values between the inbred and the outbred populations were carried out using Kruskal-Wallis and t-test, respectively.

Statistical significance tests for coefficient of variation were conducted by applying the guideline explained by Lewontin (1966). In brief, this analysis is similar to the F-test, namely by comparing the counted F-value against the distribution of F-values in the table (counted F against F table values) with corresponding degrees of freedom. With respect to the comparison of CV values, the counted F-values was obtained using Lewontin's (1966) formula: S^2_{logX}/S^2_{logY}; N_X-1; N_Y-1; where S^2_{logX} and S^2_{logY} were logarithmic transformed of morphometric variances of the first and second populations, while the N_X-1 and N_Y-1 represented the degrees of freedom of the respective populations.

Table 1. Description of twenty two morphometric characters measured within the inbred and outbred populations of *Macrobrachium rosenbergii*

Traits	Description	Ref
Rostrum length (RST)	Distance from median posterior margin to the rostrum tip	2
Head dorsal length (HDL)	Dorsal-side distance from the orbital tip to the posterior margin of cephalic carapace	*
First segment dorsal length (FDL)	Dorsal-side distance between posterior margin of cephalic carapace and posterior margin of the first abdominal segment	*
Second segment dorsal length (SDL)	Dorsal-side distance from the posterior margin of the first segment to the posterior margin of the second abdominal segment	1
Third segment dorsal length (TDL)	Dorsal-side distance from the posterior margin of the second to the posterior margin of the third abdominal segment	1
Fourth segment dorsal length (ODL)	Dorsal-side distance from the posterior margin of the third segment to the posterior margin of the third abdominal segment	1
Fifth segment dorsal length (FDL)	Dorsal-side distance from posterior of the fourth segment to the posterior margin of the fifth abdominal segment	1
Sixth segment dorsal length (XDL)	Dorsal-side distance from posterior of the fifth segment to the posterior margin of the sixth abdominal segment	1
Telson (TLS)	Distance from the tip to the base of the telson	2
Prosertema (PST)	Distance from the tip to the base of prosertema	2
Antennulles Length (UTL)	Distance from the tip to the base of antennule	

Table 1. Continued

Antenna length (ATL)	Distance from tip to the base of antenna	
Total length (TOL)	Distance from the tip of the rostrum to the tip of the telson	2
Head ventral lenth (HVL)	Ventral side distance from the base of antenna to the posterior margin of the cephalic carapace	*
First segment ventral length (FVL)	Ventral-side distance between posterior margin of cephalic carapace and posterior margin of the first abdominal segment	*
Second segment ventral length (SVL)	Ventral-side distance from the posterior margin of the first segment to the posterior margin of the second abdominal segment	*
Third segment (TVL)	Ventral-side distance from the posterior margin of the second to the posterior margin of the third abdominal segment	*
Fourth segment (FVL)	Ventral-side distance from the posterior margin of the third segment to the posterior margin of the third abdominal segment	*
Fifth segment (IVL)	Ventral-side distance from posterior of the fourth segment to the posterior margin of the fifth abdominal segment	*
Sixth segment (XVL)	Ventral-side distance from posterior of the fifth segment to the posterior margin of the sixth abdominal segment	*
Exopod (EXL)	Distance from the posterior margin of the sixth segment to the tip of the exopod.	*
Abdominal length (ABL)	Distance from the anterior margin of the abdomen to the tip of uropod	2

Notes: Reference: (1) Lester (1983); (2) Dall (1957); and single asterisk (*) new measurements developed within the current study

RESULT AND DISCUSSION

Inbreeding and the Mean of Morphometric Characters

Mean values of the 22 morphometric characters of both inbred and outbred populations are presented graphically in Figure 2 and statistical significance tests are presented in Table 2. The data presented in both the Figure 2 and Table 2 show two important features, namely a general trend of lower mean of morphometric values within the inbred population (1), and variation in the magnitude of inbreeding effect among characters (2).

Except for XVL character, all means of morphometric characters within the inbred group were lower than those of the outbred counterparts, although not all the differences were statistically significant. This general trend suggests that inbreeding depression appeared to be in place. This result is consistent with those obtained by Imron (2009) in previous study who worked on the same species but in earlier stage. He found that survival rate, the rate of developmental stage and growth of the inbred was lower than those of the outbred group.

Inbreeding depression coefficient between these two groups ranged from 3.7%-23.0%. The least and the most different characters mean values between the two groups were XVL and EXL, respectively. Further analysis by applying statistical significance test (Table 2) suggested that 36% among these mean value differences were statistically significant. The variation in morphometric characters in response to inbreeding depression suggest that the characters had a variable sensitivity in responding to the genetic make-up. Some characters were more sensitive than others. In a study aiming at investigating the effect of inbreeding on the fluctuating asymmetry and developmental stability (Mazzi *et al.*, 2002) found that the level of asymmetry in pectoral fin was higher than those in pelvic fin and gill racker.

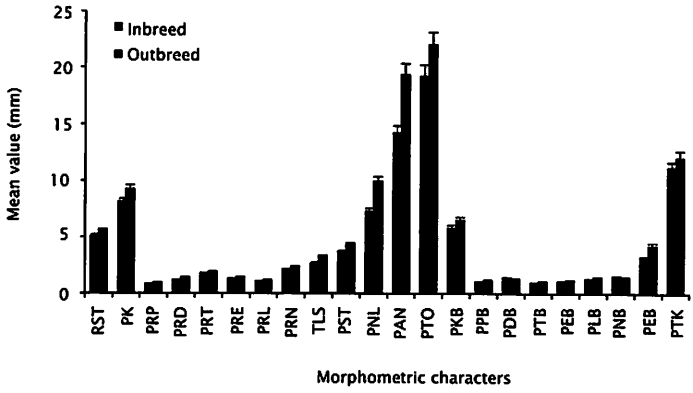

Figure 2. Profile of mean value of 22 morphometric characters of inbred and outbred groups of *Macrobrachium rosenbergii.* Abbreviation of morphometric characters along horizontal axis refer to those explained in Table 1

Table 2. Summary of mean value of twenty two morphometric characters, mean differences, and statistical significance test between populations of inbred and outbred of GFP. Ns, single asterisk and double asterisks represent statistical symbols for no significant diffrence, significant at 5% and significan at 1%, respectively

Characters	Inbreed		Outbreed		Inbreeding depression (%)	P-value	Statistical significance
	Mean	S.D.	Mean	S.D.			
RST	5.10	1.08	5.624	1.129	9.36	0.195	ns
CAL	8.14	1.52	9.098	1.499	10.57	0.034	*
FDL	0.89	0.23	0.926	0.143	3.69	0.964	ns
SDL	1.25	0.26	1.422	0.286	12.01	0.014	*
TDL	1.81	0.34	1.968	0.365	8.01	0.237	ns
ODL	1.37	0.31	1.475	0.298	7.12	0.167	ns
IDL	1.07	0.18	1.230	0.196	12.70	0.029	*
XDL	2.17	0.30	2.323	0.368	6.42	0.208	ns
TLS	2.72	0.46	3.052	0.487	11.00	0.014	*
PST	3.68	0.82	4.304	0.740	14.55	0.003	ns
UTL	7.28	3.33	9.068	3.362	19.77	0.054	ns
ATL	14.28	5.23	18.534	5.781	22.97	0.053	ns
TOL	19.33	3.22	21.488	2.866	10.04	0.031	*
HVL	5.92	1.16	6.390	1.048	7.27	0.149	ns
FVL	1.14	0.23	1.215	0.253	6.50	0.151	ns
SVL	1.44	0.34	1.474	0.367	2.35	0.405	ns
TVL	1.00	0.25	1.031	0.226	3.05	0.544	ns
OVL	1.11	0.24	1.136	0.242	2.68	0.339	ns
IVL	1.31	0.25	1.393	0.270	6.04	0.099	*
XVL	1.52	0.29	1.495	0.273	-1.42	0.705	ns
EXL	3.25	0.52	4.000	0.927	18.83	0	**
ABL	11.23	1.85	12.812	1.326	12.36	0.045	*

Inbreeding and Phenotypic Variation

In contrast to the relatively significant effect of inbreeding on the mean of morphometric characters, its effect on the phenotypic variation, as expressed as CV values, was generally not quite substantial. Although in majority of the characters inbred shows a slightly higher CV (Figure 3), their magnitude are not significantly different from those of the outbred (Table 3). Despite insignificant, the observed pattern in which inbred's CV were slightly higher than those of the outbred's counterpart, was actually conform with that predicted under homeostasis theory in which developmental instability increase with inbreeding (Deng, 1997).

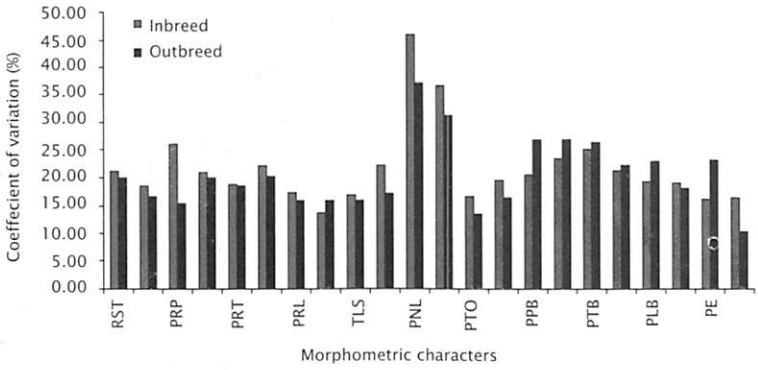

Figure 3. Profile of coeffecient of variation (CV) across 22 morphometric characters of inbred and outbred populations of *Macrobrachium rosenbergii*. Abbreviation of morphometric characters along horizontal axis refer to those explained in Table 1

Tabel 3. Summary of CV value of morphometric characters within the inbred and the outbred groups and their statistical significance values

Character	CV of inbred (%)	CV of outbred (%)	S2log(I)/ S2log(O)	Statistical significance
RST	21.125	20.069	0.611	NS
CAL	18.658	16.472	1.032	NS
FDL	26.013	15.424	0.751	NS
SDL	21.097	20.100	1.064	NS
TDL	18.747	18.526	1.071	NS
ODL	22.261	20.224	0.982	NS
IDL	17.218	15.975	1.037	NS
XDL	13.585	15.859	1.221	NS
TLS	16.802	15.974	1.092	NS
PST	22.180	17.186	0.675	NS
UTL	45.827	37.072	0.993	NS
ATL	36.647	31.189	0.943	NS
TOL	16.681	13.340	1.112	NS
HVL	19.622	16.396	3.237	**
FVL	20.494	20.801	1.060	NS
SVL	23.404	24.891	1.085	NS
TVL	25.086	21.884	0.929	NS
OVL	21.300	21.326	1.020	NS
IVL	19.368	19.347	1.047	NS
XVL	19.103	18.264	0.954	NS
EXL	16.120	15.794	1.363	NS
ABL	16.440	10.351	2.172	**

Implications for Selective Breeding Program

The objective of selective breeding program is to improve performance in the character of interest through genetic gain over generations. With respect to genetic gain, two parameters of quantitative genetic, namely differential selection and heritability, determine population's ability to respond to selection (Falconer & Mackay, 1996). Genetic gain or response to selection is a product of those two parameters, as expressed in the equation R=h2S; where R, h2, and S represent for response to selection, heritability, and differential selection, respectively. The maximum response of a trait to selection will be achieved when a population is characterized by a high differential selection and heritability. While heritability estimate of both populations need to be investigated further, the current data, particularly mean and CV of morphometric characters, may give insight as to the magnitude of differential selection potential.

Differential selection is a difference between the maximum or minimum values and the corresponding population mean value of a given character within a breeding population. Assuming heritability values of a given character in both populations are similar, a higher differential selection within a population means the likely to gain higher selection response is also higher. With a few exceptions, the data obtained in the current study of variety suggested two important features: lower level of mean and a slightly higher level of coefficient of variation in the inbred group than those of the outbred. Under circumstances of equal morphometric means, population with a higher CV will have a wider range of phenotypic values, which means higher potential of differential selection. However, this was not the case with the inbred group. In addition to the lack of significant magnitude of CV differences, the inbred also were characterized by lower values in both mean and ranges of morphometric values. Combination of these properties implies that the inbred groups have a lower potential of differential selection. Consequently, the use of inbred group as broodstock within selective breeding program will prevent the breeding program from obtaining maximum response to selection. Letting the inbreeding to occur generation after generation will result in the accumulation of inbreeding coefficient to a level that may deteriorate performance.

Despite the quite convincing results, it should be noted that the present data is of preliminary in nature due to limited scope of life stage. Whether the pattern observed in the current study will remain consistent at later stage, e.g. at marketable size by which time the selection for trait of interest taken place, need to be explored further. To address this query, the life specimens of both groups are currently being maintained for further observations.

CONCLUSION

Inbreeding significantly reduced survival of *Macrobrachium rosenbergii* during post larval and first nursery stages. The effect on nursery stage was more significant than that on post larval stage. Inbreeding also reduced growth in the form of lower level of morphometric variability means and increased phenotypic variability within the inbred implying the use of it for selective breeding is susceptible of yielding a lower response to selection. Over all, inbreeding coefficient within breeding population need to be controlled that the adverse effect of inbreeding can be minimized.

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