ASSOCIATION OF MICROSATELLITE GENETIC DIVERSITY WITH GROWTH RELATED TRAITS IN THE BASE POPULATION OF AFRICAN CATFISH, *Clarias gariepinus*, BREEDING PROGRAM

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ABSTRACT

Genetic diversity at molecular level has been assumed to correlate with fitness related traits. However, accumulating evidences showed that the nature of that correlation has been variable. This study was aimed to explore the nature and possible mechanisms underlying that correlation by focusing on growth related traits in African catfish, *Clarias gariepinus* using microsatellite molecular markers. Fifty individual African catfish of 110 days-old were sampled and subjected to both morphological and molecular analyses. The standard length, total length and body weight as well as allelic scores of six microsatellite loci were measured on each individual. Indices of microsatellite diversity, namely individual multilocus heterozygosity (MLH) and mean microsatellite allelic distance (mean d2) for individual level, and mean observed heterozygosity (*Ho*) and single-locus heterozygosity (*ho*) for group level, were correlated to those traits using Pearson correlation coefficient (*r*). The Hardy-Weinberg and linkage disequilibrium were carried out to explore the possible mechanisms underlying correlation. The results showed that at individual level the MLH and mean d2 were weakly correlated with standard length, ($r=0.25$, $p<0.05$) and ($r=0.24$, $p<0.05$), respectively. At group level, *Ho* was correlated with both standard and total length ($r=0.99$, $p<0.05$) while *ho* identified two loci, *Cga*03 and *Cga*06 significantly contributed to the correlation. Combining all relevant information, present study identified associative overdominance, both local effect and general effect hypotheses might responsible for the observed correlations.

KEYWORDS: **microsatellite genetic diversity, multilocus heterozygosity, microsatellite allelic distance, observed heterozygosity, African catfish, *Clarias gariepinus***

INTRODUCTION

Genetic diversity detected at molecular level is widely assumed to correlate with phenotypic performance. This idea, commonly called as genetic diversity-fitness correlation (GDFC) (Da Silva *et al.*, 2006), has long been argumentative (Reed & Frankham, 2001) as accumulating empirical evidences have not always been consistent. Although most studies favour for the positive correlation (reviewed by Reed & Frankham, 2003), other studies (e.g. Del Rio-Portilla & Beaumont, 2000) and meta-analysis studies (Chapman *et al.*, 2009; Heath *et al.*, 2002) found a weak or non significant relationship. This variation seems to associate with various aspects such as genetic background of populations (Del Rio-Portilla & Beaumont, 2000; Saavedra & Guerra, 1996), life stages (Pierce & Mitton, 1982), traits being investigated (Heath *et al.*, 2002), and molecular marker as well as diversity indices in use.

With respect to genetic markers, allozyme have dominated the study of GDFC. In reviewing this subject through meta analysis, Reed & Frankham (2001) reported that over 70 percent studies on GDFC, have been done using allozyme markers. In recent years however, with the advance in molecular tools, particularly the polymerase chain reaction (PCR), the trend has been changing toward the use of microsatellite markers. These markers have advantages over allozymes as they are highly polymorphic, codominant, and selectively neutral, making it marker of choice for many genetic studies, including the studies of GDFC (Wright & Bentzen, 1994).

Several measures of genetic diversity indices generated by microsatellite marker exist, some of which are heterozygosity (H), and microsatellite allelic distance (d2) (Primmer & Tiira, 2009). Heterozygosity

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can be analysed in two levels, namely group and individual levels. At group level, heterozygosity is defined as the proportion of heterozygote at all loci divided by all loci sampled (Frankham *et al*., 2002). At individual level, heterozygosity refers to the number of heterozygous allele within each individual. Heterozygosity at this level has commonly been called as individual heterozygosity (Appleyard *et al.*, 2001; Mitton & Pierce, 1980), or multilocus heterozygosity (MLH) (Chapman *et al.*, 2009; Diehl & Biesiot, 1994; Kittiwattanawong, 2001; Rolan-Alvarez *et al.*, 1995; Slate *et al.*, 2004). For the purpose of consistency we use this latter term throughout this paper. Another index of microsatellite genetic diversity is squared allelic distance (d2). It represents the square of the difference in repeat units between the two alleles at a locus, and it assumes a stepwise mutation model. The latter measure was recently introduced (Coulson *et al*., 1998) and has been considered as a good indicator for capturing fitness effect of both ends of inbreeding-outbreeding spectrums (Hansson, 2010). Its use in the study of genotype-fitness correlation has been documented, for instance in chinook salmon, *Oncorhynchus tshawytscha* (Heath *et al.*, 2002) and harp seals, *Phoca groenlandica,* (Kretzmann *et al.*, 2006).

There have been at least three prevailing theoretical explanations for the GDFC. The first is direct overdominance hypothesis, where the scored loci might have a direct effect on individual fitness (Zouros *et al.*, 1980). The second is associative over dominance in which linkage disequilibrium might result in association between marker loci and fitness loci (David *et al.*, 1997). The latter hypothesis can be split further into local effect and general effect hypotheses (Liu *et al.*, 2006). While the former occurs when fitness loci rest in the local surrounding chromosomal region, the latter occurs when heterozygosity at the markers might reflect variation in inbreeding coefficient and might associate with fitness as result of homozygosity at genome-wide distributed loci (Hansson & Westerberg, 2002).

African catfish, C*larias gariepinus* have been one of the highly popular food fish and has contributed significantly to the program of food security in Indonesia. Breeding program aiming at improving growth rate of this species has been started in 2011 at the Research Institute for Fish Breeding (RIFB) and a base population consisting of reciprocal crossing of four strains was established.

This study was designed to address a general question on the GDFC. Specifically, it was intended to explore several specific questions, namely: 1) whether correlation between genetic diversity and growth exist in captive population of African catfish, 2) the pattern and magnitude of genetic diversity-fitness correlation, 3) if the correlation exists, which parameter of genetic diversity indices could serve best as surrogate for fitness trait. Finally, it was also intended to explore which hypothesis, among the three, that may best explain the GDFC in captive population of African catfish. Those three questions were explored within the base population of African catfish breeding program using microsatellite molecular markers.

MATERIALS AND METHODS

Sample

Sample was a base population of African catfish breeding program at Research Institute for Fish Breeding (RIFB). It was a composite population made up of reciprocal mating among four geographic populations that were already distributed and widely used by catfish farmers. These founding populations were Sangkuriang, Dumbo, Paiton, and Egypt strains. Except the last one, the history and genetic attributes of the other three strains, as revealed by microsatellite markers, have been described by Imron *et al.* (2011). Fifty individual were randomly sampled at harvest (110 days old) and were used for growth-related trait measurement and microsatellite genotyping.

Growth-Related Traits Measurement

Growth-related traits assessed were standard length, total length and body weight. Standard length was measured to the nearest 1 mm using a plastic ruler, while body weight scaled to the nearest 0.2 g using Kern electric balance. Following these phenotypic measurement, 20-50 mg muscle tissue was taken from each individual sample for microsatellite analysis.

Microsatellite Genotyping

Microsatellite analysis was performed using six polymorphic loci (Galbusera *et al.*, 1996) the description of which are described in Table 1. Genomic DNA was extracted from caudal fin of each the individual of African catfish. All extractions were performed by following the manufacturer's instructions (*Gentra puregene, Qiagen*). The protocol consists of series of steps including cell lysis, protein precipitation, DNA precipitation, washing and DNA hydration. Cell lysis was performed by weighing of approximately 10 mg of tissue sample and mincing it with a surgical blade into small pieces. The tissue was lysed by adding 300 µL of cell lysis solution into a 1.5 mL microcentrifuge tube, 1.5 µL of puregene proteinase K, followed by incubation in 55°C for 3 hours and adding 1.5 µL of RNase A solution followed by incubation in 37°C for 15 min. The lysate was added 300 µL with isopro-

Table 1. Description of six microsatellite primers (Galbusera *et al.*, 1996) used to amplify polymorphic microsatellite alleles in the base populations of *Clarias gariepinus* breeding program

Locus	Gene bank accession number	Primer sequence (5'-3')	Size range of PCR product (bp)	TM (^{o}C)
Cga 01	U30862	F: GGCTAAAAGAACCCTGTCTG R: TACAGCGTCGATAAGCCAGG	92-104	59
Cga 02	U30863	F: GCTAGTGTGAACGCAAGGC R: ACCTCTGAGATAAAACACAGC	102-110	58
Cga 03	U30864	F: CACTTCTTACATTTGTGCCC R: AACCTGTATTGATTTCTTGCC	142-168	56
Cga 05	U30866	F: TCCACATTAAGGACAACCACCG R: TTTGCAGTTCACGACTGCCG	204-212	60
Cga 06	U30867	F: CAGCTCGTGTTTAATTTGGC R: TTGTACGAGAACCGTGCCAGG	134-142	60
Cga 09	U30871	F: CGTCCACTTCCCCTAGAGCG R: CAGCTGCATTACCATACATGG	180-196	65

TM = Annealing temperature

panol followed by vortexing for 50 seconds and then spinning at 16,000 x*g* for 1 min. The supernatant was discarded and the pellet remains was taken care in the tube. The pellet DNA was then washed by adding 300 µL of 70% ethanol, inverting several times and spinning at 16,000 x*g* for 1 min by microcentrifuge. The supernatant was discarded carefully and adding 100 µL DNA hydration solution and vortexing for 5 seconds at medium speed. Finally, the dissolved the DNA was incubated at 65°C for 1 hour. To check the success of genomic DNA extraction process, the sample was run on mini horizontal gel electrophoresis. The sample was loaded in to the 1% (w/v) agarose gel, powered with 65 volt electricity and run for 70 minutes and viewed using gel documentation system ultraviolet transillumination. The purity and quantity of DNA was measured using a GeneQuant 1300.

PCR Amplification and Genotyping and Allele Scoring

A total of 2 mL (7200 ng) of extracted genomic DNA was amplified by using type-it microsatellite PCR (Qiagen). Amplification of polymorphic microsatellite locus was performed using thermal cycling system (mycycler, Biorad). The PCR process was done with the program: initial denaturing at 95°C for 5 min; (denaturing at 95°C for 30 seconds; annealing at 50°C-62°C for 90 seconds depending on annealing temperature of each primer (Table 1); extension at 72°C for 30 seconds) for 30 cycles; and final extension 60°C for 30 minutes. PCR amplification results were checked by electrophoresis on 2% agarose gel that has given dyes GelRedTM (Biotium) and electrophoresis results were observed under UV light and photographed using transluminator digital camera Canon EOS 1100D.

Polymorphism of microsatellite locus were screened using a fragment analysis tool QIAxcel (Qiagen) using DNA QIAxcel Screening kit (Qiagen), the size of the alleles was determined based on the relative size of the PCR products with reference to the size QX DNA marker 50-800 bp size marker (Qiagen). Electropherogram banding pattern data were analysed using QIAxcel ScreenGel software (Qiagen) for scoring alleles. The allelic data were then analysed further to generate various genetic parameters.

Parameters and Data Analysis

Four microsatellite genetic diversity indices were used to assess the association between genetic diversity and growth related traits. They were multilocus heterozygosity (MLH), mean microsatellite allelic distance (mean d2), and observed heterozygosity which was examined as mean observed heterozygosity (*Ho*) and single locus observed heterozygosity (*ho*). The MLH and mean d2 are genetic diversity parameters treating individuals as study unit. In this way, each individual possesses observational value according to study design. In the current study, each individual had both record of growth related trait values and molecular data allowing correlation analysis to be done on individual basis. In contrast to this, observed heterozygosity (*Ho* and *ho*) treat group as study unit. The following are mathematical expressions for obtaining those genetic diversity indices.

MLH*i* = N heterozygous (*i*) / N genotyped (*i*), that was the proportion of heterozygous microsatellite

loci over all loci that have been successfully genotyped within each individual sample.

Mean microsatellite allelic distance (mean d2):

$$\text{Mean d2} = \sum_{i=1}^{n} \{(ia - ib)^2 / n\}$$

where:
ia and *ib* are the length of repeat unit of allele *a* and *b* at locus *i* and *n* is the number of typed loci (Hansson, 2010).

Mean observed heterozygosity (*Ho*) = Total number of heterozygotes averaged over all loci / Sample size (Frankham *et al.*, 2002)

Single-locus observed heterozygosity (*ho*) = Total number of heterozygotes at single locus / Sample size (Frankham *et al.*, 2002)

Microsatellite allelic data were analysed using FSTAT (Goudet, 2001) to obtain relevant genetic diversity parameters, particularly *Ho*, *ho* and fixation index, F*is*. The patterns and magnitude of GDFC were assessed using correlation analyses. To obtain an insight on the relationship between genetic diversity and growth related traits, correlation analyses were explored in two levels, namely individual and group. Individual-based correlation analysis was carried out by correlating the phenotypic and molecular data owned by each individual sample. Group-based correlation analysis was carried out through two steps; firstly, grouping individual sample into three size groups, followed by correlating the average values of both molecular marker and phenotypic traits within the respective groups.

Grouping of samples into three groups based on its size, namely small (S), medium (M), and large (L) was conducted based on the following quantitative criteria. The S group were individuals with phenotypic value smaller than sample average minus one standard deviation (SD). Mathematically, it was expressed as (Y < X-1SD), where Y was observation value and X was population average. The medium-size group were those individual with phenotypic values falling within sample average plus/minus one SD ((X+1SD) > Y > (X-1SD)), and the L group were those with the value of phenotypic measurement larger than sample average plus 1 SD (Y > X+1SD). By applying these criteria, the distribution of 50 individual samples within the respective group is presented in Table 2. Statistical test contrasting the mean between groups (results not shown) found that they were significantly different ($p<0.05$).

Direction or trend of the correlation, whether it is positive, negative or uncorrelated, was reflected in the sign of Pearson correlation coefficient (*r*) which could be either positive, negative, or zero. In graphical illustration this was visualized in the slope of trend line. The magnitude of the correlation was expressed in the *r*-value, and its statistical significance was tested at $\alpha = 0.05$. Null hypothesis (H0) of no correlation between microsatellite genetic diversity and growth related traits was tested against the alternative hypothesis (H1), in which correlations exist, either in positive or negative direction. All the analyses were performed in excel add-in *analyse-it*.

To address question on the possible mechanisms underlying correlation, if any, was explored in several ways: Hardy-Weinberg test, linkage disequilibrium, and single locus correlation analyses. Output of the analyses, namely the pattern of either heterozygote deficit or heterozygote excess, was then contrasted to prevailing hypotheses that were previously described, namely direct over dominance, local effect, and general effect hypotheses. Data analysis was done using genetic software *Genepop* (Raymond & Rousset, 1995), *Arlequin* (Schneider *et al.*, 2000), and FSTAT (Goudet, 2001).

RESULTS

Correlation of Multilocus Heterozygosity with Growth-Related Traits

The relative value of MLH calculated over six loci ranged from 0 to 0.83. This mean that at one end

Table 2. Grouping of sample into small (S), medium (M), and large (L) groups on the bases of standard length. The Y, X and N indicates observation value, sample average, and number of sample, respectively

Group	Criteria	Range of standard length (cm)	N
Small	Y < X-1SD	19.1-21.2	9
Medium	(X+1SD) > Y > (X-1SD)	21.9-23.4	32
Large	Y > X+1SD	28.5-33.0	9
Total			50

several individuals had no loci with heterozygous allele (MLH = 0) while at the other end, individuals had maximum 5 loci of heterozygous alleles (MLH = 0.83). However, as can be seen in Figure 1, the number of individuals reside within both ends was scarce (less than five percent). Majority of the sample had 1-4 loci of heterozygous alleles. Figure 1 shows the direction and magnitude as well as statistical significance of correlation between this genetic diversity index and growth related traits. In general, there was positive correlation between MLH and growth related traits. This was shown by the slope of trend lines pointing toward up-right in plotting of MLH against standard length, total length and body weight (Figure 1). Despite positive, however, degree of correlations between MLH and growth related traits were relatively weak. The value of correlation coefficient (r) for MLH-standard length, MLH-total length, and MLH-body weight were 0.25, 0.22 and 0.20, respectively. Among these, only the correlation of MLH-standard length that was statistically significant ($p<0.05$).

Correlation of Mean d2 to Growth Related Traits

In line with MLH, the mean microsatellite allelic distance (mean d2) was positively correlated with growth related traits. The slope of trending line which toward up-right lend a support for this trend of correlation. However, the strength of correlation was also weak. They ranged from 0.19 ($p>0.05$) for body weight to 0.24 ($p<0.05$) for standard length (Figure 2). Under mean d2 perspective, more heterozygous individuals would have larger allele differences. This figure suggest that more homozygous individuals, namely those with less value of allele differences, performed poorer in all growth related traits than those with more distant allele difference.

Correlation of Mean and Single Locus Heterozygosity with Growth Related Traits

In contrast to those observed at individual level, association analyses conducted at group level, showed more significant correlation. Pearson coefficient correlation between mean observed heterozygosity and both standard and total lengths was 0,99 ($p<0.05$), respectively, while that for body weight was 0,98 ($p>0.05$) (Table 2). This pattern of correlation means that there was a tight relationship when the samples were classified into small, medium and large groups, followed by correlating the mean phenotypic value of each group to the respective mean of the observed heterozygosity. The samples belonging to the small-size group were found to have smallest mean heterozygosity. Likewise, group of medium and large size were found to have medium and largest observed

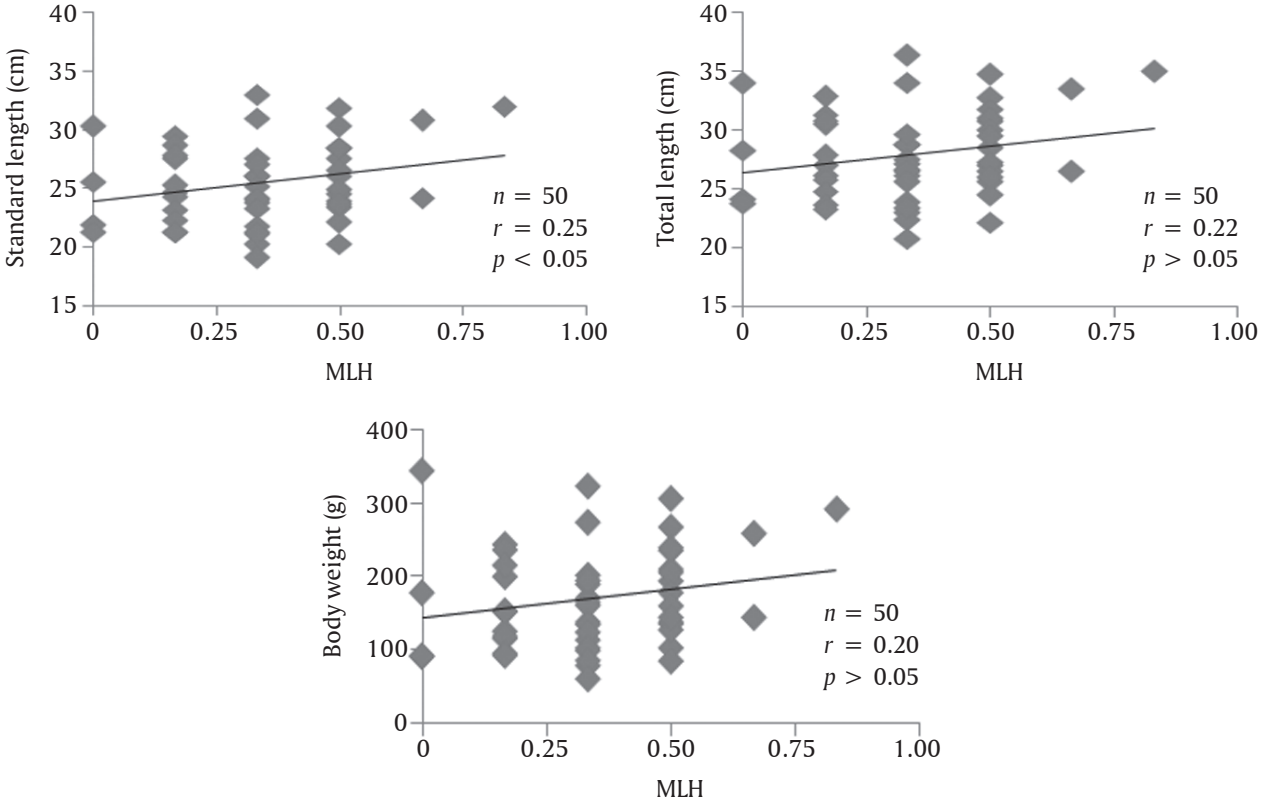

Figure 1. Association between multilocus heterozygosity (MLH) and growth-related traits, including standard length (A), total length (B) and body weight (C). The n, r, and p indicate number of sample, Pearson correlation coefficient, and statistical significance, respectively

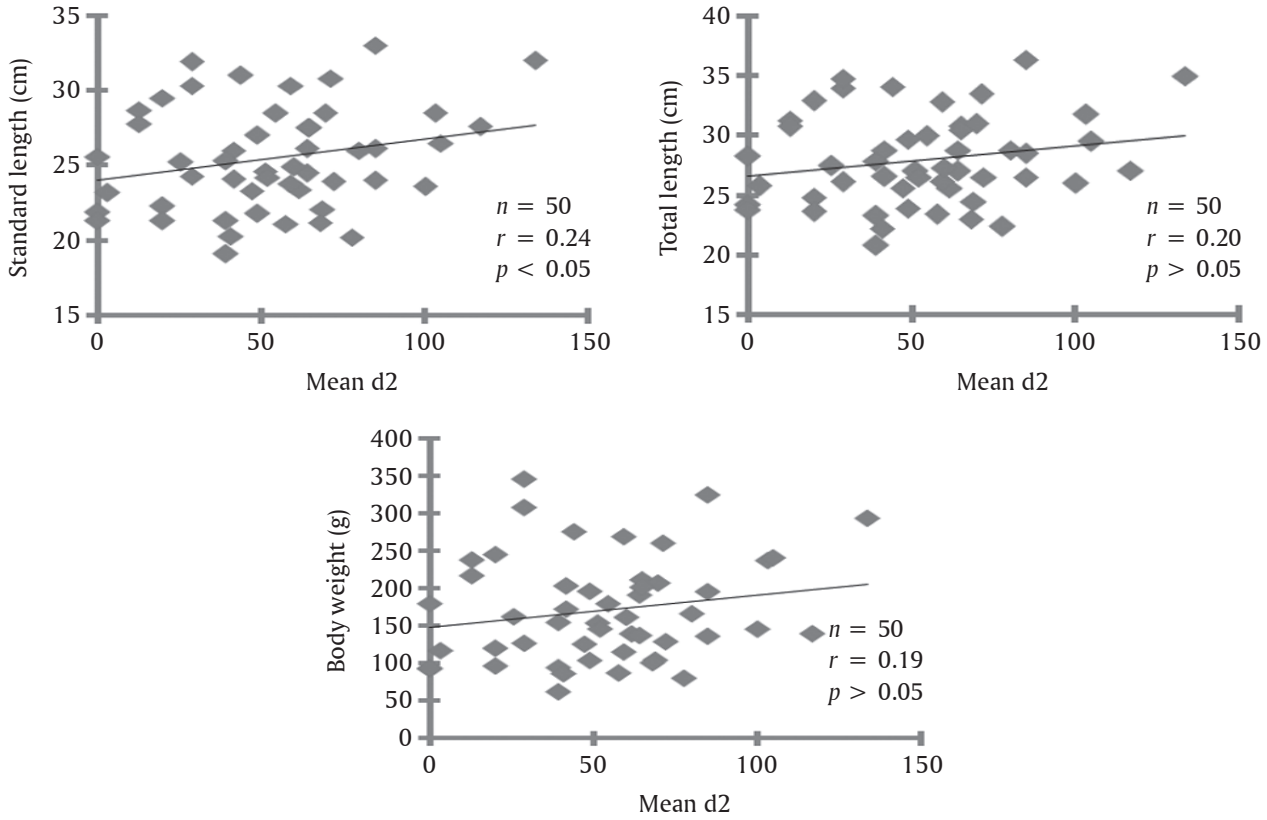

Figure 2. Association between mean d2 and growth related traits including standard length (A), total length (B) and body weight (C). The n, r, and p indicate number of sample, Pearson correlation coefficient, and statistical significance, respectively

heterozygosity, respectively. The figure seems to show that mean observed heterozygosity appeared to be more sensitive in detecting genetic diversity-growth related trait association.

While mean observed heterozygosity showed positive correlation, analysis on single locus showed that not all loci contributed in a similar way to the trend and degree of association. Out of six loci analysed, only two loci, namely *Cga*03 and *Cga*06 that were positively and significantly correlated ($p<0.05$) with growth-related traits (Table 3). Trend and degree of correlation of the other loci, were positive, negative or uncorrelated, but they were all statistically not significant ($p>0.05$) (Table 3).

Profile of Heterozygote Deficit and Linkage Disequilibrium

Analyses of Hardy-Weinberg equilibrium presented in Table 3 showed that all loci significantly deviated from Hardy-Weinberg equilibrium. Specifically, all loci experienced heterozygote deficit and all were statistically significant ($p<0.05$). This was observed in both single locus and combined loci analyses. Analysis of linkage disequilibrium (Table 5) showed that one pair loci (*Cga*02 and *Cga*06) was in disequilibrium ($p<0.05$) while other loci pairs were in equilibrium ($p>0.05$). This pattern was in line with the result of correlation between single-locus heterozygosity and growth related traits previously presented (Table 4).

DISCUSSION

Exploration on GDFC carried out in this study resulted in several interesting findings. They were: 1) the existence of the signal of correlation, 2) similarity in power between MLH and mean d2 as indicator in GDFC studies, 3) similarity in direction but not in magnitude between correlation profiles resulted from individual and those from group bases, 4) variation in magnitude of correlations among different measures of growth related traits, and 5) local and general effect hypotheses seemed to involve in the emergence of GDFC phenomena observed in this study. The following discussion describes those findings in detail.

The Nature of GDFC in The Base Population of African Catfish Breeding Program

The MLH has long been used as genetic diversity parameter in studies of GDFC. In contrast to this, mean d2 has been only recently introduced (Coulson et al., 1998). Present study found that both MLH and mean d2 gave similar results, namely weak, but posi-

Table 3. Direction, magnitude, and statistical significance of correlation between both single locus (*ho*) and mean (*Ho*) observed heterozygosity and growth related traits in 110-days old of African catfish base population. Sample size = 3, degree of freedom = 1

Traits	Locus	Heterozygosity		r-statistics	*p*-value at H1; r ≠ 0
		ho	*Ho*		
Standard length	Cga01	0.78		-0.89	0.3002
	Cga02	0.55		-0.07	0.9574
	Cga03	**0.33**		**1.00**	**0.0311**
	Cga05	0		-0.85	0.3554
	Cga06	**0.67**		**1.00**	**0.0426**
	Cga09	0.67		0.92	0.2552
	All loci combined		0.311	0.99	<0.05
Total length	*Cga* 01	0.81		-0.89	0.3081
	Cga 02	0.53		-0.08	0.9495
	***Cga* 03**	**0.21**		**1.00**	**0.0390**
	Cga 05	0.13		-0.85	0.3475
	***Cga* 06**	**0.50**		**1.00**	**0.0505**
	Cga 09	0.56		0.93	0.2474
	All loci combined		0.311	0.99	<0.05
Body weight	*Cga* 01	1.00		-0.85	0.3484
	Cga 02	0.44		-0.14	0.9093
	Cga 03	0.11		0.99	0.0792
	Cga 05	0.11		-0.89	0.3092
	Cga 06	0.22		0.99	0.0907
	Cga 09	0.56		0.95	0.2071
	All loci combined		0.311	0.98	>0.05

tive and statistically significant correlations of both of them and standard length. This profile of correlation may reflect several indications. Firstly, within the population under study, the GDFC existed. This supports widely accepted thought and provides additional supporting evidence. Secondly, it lends some support that inbreeding may be involved in the emergence of the GDFC phenomena in this population. This argument is based on the proposal that mean d2 is considered as good indicator of individual inbreeding level (Hansson, 2010). Therefore, significant correlation between this parameter and growth related traits suggest that inbreeding could be involved as a causing agent underlying the GDFC within this study. Special discussion exploring the possible mechanisms underlying GDFC, described later in this paper, strengthening this proposition.

The lower magnitude of GDFC at individual level detected in this study could be explained because microsatellite loci are neutral marker giving no direct effect on the expression of fitness traits. Rather, it may work indirectly through association with loci affecting the fitness traits. Hence, degree of association between marker loci and fitness loci may determine the strength of association between marker loci and fitness traits. The weak association observed in this study might result from the situation in which marker loci were less associated with fitness loci. This was supported by the fact that based on single locus analysis, only two loci (*Cga*03 and *Cga*06) which were significantly correlated with growth traits. The weak correlation at individual level detected in this study (r ranged from 0.19 to 0.25), albeit small, is within the range of previous studies. Bierne *et al*. (2000) for instance, found a weak but significant and positive correlation between microsatellite tri-locus heterozygosity and growth rate ($r=0.3$, $p=0.02$). This relatively weak association was in contrast to those obtained at group level.

The relatively stronger association at group level suggest that overall heterozygosity, may have more important role in fitness trait. It also might indicate that population understudy had experienced some level of inbreeding depression. A comparable study

Table 4. Profile of heterozygote deficit at six loci calculated on both single locus and combined bases. The *ho*, *Ho*, *he* and *He* refer to single-locus observed heterozygosity, mean observed heterozygosity, single-locus expected heterozygosity, and mean expected heterozygosity, respectively. Single and double asterisks next to p-value indicate statistical significance at $\alpha = 0.05$ and $\alpha = 0.01$, respectively

Locus	Observed heterozygosity		Expected heterozygosity		*p*-value
	ho	*Ho*	*he*	*He*	
Cga01	0.78		0.913		0.0426*
Cga02	0.55		0.820		0.0000**
Cga03	0.33		0.735		0.0000**
Cga05	0		0.795		0.0000**
Cga06	0.67		0.833		0.0000**
Cga09	0.67		0.916		0.0000**
All loci combined		0.311		0.83878	0.0000**

Table 5. Test of linkage disequilibrium over fifteen pairs loci. The Chi2, df, p-value indicate the value of chi square, degree of freedom and statistical significance, respectively

Locus pair	Chi 2	df	*p*-value
*Cga*01 & Cga02	4.051119	4	0.399132
*Cga*01 & Cga03	7.812147	4	0.098707
*Cga*02 & Cga03	5.815778	4	0.444140
*Cga*01 & Cga05	0.486463	4	0.974805
*Cga*02 & Cga05	2.972427	6	0.812299
*Cga*03 & Cga05	1.707876	6	0.944512
*Cga*01 & Cga06	1.059439	4	0.900653
*Cga*02 & Cga06	13.497239	6	0.035785*
*Cga*03 & Cga06	2.457263	6	0.873220
*Cga*05 & Cga06	3.010361	6	0.807545
*Cga*01 & Cga09	0.000000	4	1.000000
*Cga*02 & Cga09	3.051297	4	0.549278
*Cga*03 & Cga09	1.275690	4	0.865486
*Cga*05 & Cga09	5.781304	4	0.216087
*Cga*06 & Cga09	0.933522	4	0.919703

that in line with the present result came from Shikano and Taniguchi (2002) who worked with guppy, *Poecilia reticulata,* of different genetic background to examine the effect of genetic diversity on salinity tolerance. They found that at individual level salinity tolerance did not correlate with the multilocus heterozygosity or with mean d2, while at population level salinity tolerant was positively correlated with the mean heterozygosity. The results from group-level analyses obtained from the current study, despite provide strong signals of HDFC need to be treated carefully due to limited sample size used to generate the picture. Hence, it could only be considered as prospective preliminary information and should be used with a high caution.

Possible Causes of GDFC and Practical Implications for Breeding Program

Results of present study, as previously described, show that the relationships exist, and it is of interest to explore based on available data, the mechanisms underlying the emerging phenomena. Specifically, we examine the phenomena under three major GDFC hypotheses: direct-effect over dominance, lo-

cal-associative over dominance, and general-associative over dominance.

The direct-effect over dominance hypothesis views that GDFC occurs due to direct effect of microsatellite loci to the fitness traits. However, this hypothesis is less likely to hold due to the widely accepted idea that microsatellite loci are selectively neutral marker (O'Malley *et al.*, 2013), meaning that there is no direct effect of the loci to any fitness traits. Based on this argument, direct effect over dominance hypothesis could be ruled out (Bierne *et al.*, 2000), and leaving associative over dominance hypothesis as possible underlying mechanism. Under associative over dominance hypotheses, fitness loci could be rest in either particular chromosome which is called local effect, or they are distributed in genome-wide known as general effect. Indicators that could be assessed to differentiate between the two hypotheses are the pattern of inbreeding reflected in heterozygote deficit (or homozygote excess) and linkage disequilibrium. Under local effect hypothesis, the heterozygote deficit occurs limited to particular chromosomes, while under general effect hypothesis, heterozygote deficit is distributed in the whole genome (David, 1998). With respect to linkage disequilibrium, local effect hypothesis would be reflected in the presence of statistical significance of several pairs of loci. In the present study, the data support both local and general effect hypotheses.

Support for local effect hypothesis came from single-locus heterozygosity (Table 4) and genotype disequilibrium (Table 5) analyses. The fact that only two loci (*Cga*03 and *Cga*06) that are positively correlated, and supported by disequilibrium state of those loci pairs in linkage analysis, provided supports for that hypothesis. However, that hypothesis appeared to be not the only explanation. General effect hypothesis seemed also appropriate to explain the mechanism underlying the observed GDFC, as the heterozygote deficit occurred within all loci. Assuming the loci being used are representative of the genome, it is plausible to conclude that genome-wide homozygosity as result of inbreeding as mechanism responsible for the observed GDFC in the base population of African catfish breeding program. This explanation is reasonable given the fact that population understudy are offspring of individual fish which were artificially bred in captivity. It is right that this base population are a composite population consisting of reciprocal crossing of 4 strains, which in theory inbreeding could be minimized. However, limited number of broodstock used in spawning did not allow to completely prevent inbreeding from happening. Furthermore, pooling of male gametes during artificial spawning carried out in this study, might result in differential reproductive success which lead to decreased effective breeding number and increased population's inbreeding rate.

The phenomena observed in this study where local and general effects appeared to be involved in generating the existence of GDFC were resemble to those obtained by Heath *et al.* (2002) who worked with chinook salmon. They found that while majority of relationships were found to be nonsignificant, one microsatellite locus, namely *Omy27*, was significantly correlated with reproductive fitness. Based on those findings they concluded that both local and general effects support for the existence of GDFC in chinook salmon. Independent of the mechanisms underlying GDFC, one thing that is obvious in this study is that positive correlation existed between genetic diversity and growth related traits in the base population of African catfish breeding program. Eventually, for practical purposes, it is important to see what these findings may imply for aquaculturists and breeders.

For aquaculture practitioners and breeders it is of important to have fish population having good fitness in either productive or reproductive traits. This study provides information that individuals or groups with higher genetic diversity as expressed in various indicators, proved to have higher growth related traits. For breeders, it is a clear message that maintaining a high level of genetic diversity is crucial. This finding provides support for a generally accepted proposition to maintain a high level of genetic diversity, particularly heterozygosity in a breeding population.

CONCLUSIONS

There was positive correlation between various measures of microsatellite genetic diversity and growth related traits in the base population of African catfish breeding program. The signal of correlation was weakly detected at individual level, while stronger signals were detected at group level. The *Cga*03 and *Cga*06 appeared to be the most contributed loci to the presence of GDFC in African catfish and deserve further exploration to take benefit for breeding program. Associative over dominance, both local effect and general effect hypotheses, were equally possible in explaining the phenomena of GDFC.

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