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Microsatellite loci heterozygosity and fitness correlations among three genetic groups of domesticated mallard ducks (*Anas platyrhynchos domesticus* L.) in the Philippines

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The relationships between the degree of multi-loci heterozygosity in the Philippine Mallard, Khaki Campbell, and the Pekin duck populations based on 21 polymorphic microsatellites and the bodyweight, breast depth, breast width, keel length, and shank length at maturity were determined. The multi-loci heterozygosity was highest in the Pekin with 0.3985 followed by that of the Khaki Campbell and Philippine Mallard with 0.3077 and 0.2711, respectively. In addition, significant correlations were detected between the microsatellite loci heterozygosity and bodyweight at maturity (r = 0.468), breast width (r = 0.410), breast depth (r = 0.443) and keel length (r = 0.449) of the mallard ducks in an overall population basis. This suggests that the individual performance potential of the ducks in these traits could be ascertained early in their life based on the degree of their individual heterozygosity by employing linear regression analysis.

Keywords: Heterozygosity fitness correlations, Microsatellites, Philippine Mallard duck

Introduction

In the Philippines, duck ranks next to chicken in economic importance as source of eggs and poultry meat. The local duck industry however is being faced by a very pressing problem on the lack of quality breeder stocks (Chang *et al.*, 2003; Lambio, 2009). Improving the genetic make-up of the stocks being raised by the local duck farmers can sustainably enhance their production efficiency. In designing and in carrying out sustainable breeding programs, looking into the correlations between individual microsatellite loci heterozygosity and fitness traits could provide a guide towards a more rapid genetic improvement of the mallard ducks in the country.

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A common way to study the interactions between genetic diversity and fitness is to look at heterozygosity-fitness correlations (HFCs) or the empirical observation of a correlation between heterozygosity measured at a marker locus, or at a set of marker loci, and a fitness-related trait (Gosselin and Bernatchez, 2006). According to Liu *et al.* (2006), HFCs are not only of theoretical interest as they are also valuable in animal breeding. Determining the multi-loci heterozygosity of an individual could be done as early as possible using molecular markers such as the microsatellites. The detection of a significant correlation between the microsatellite multi-loci heterozygosity and the fitness traits of an animal would be of great help in predicting the fitness potential of the individual at its early life stage. This will make the selection process for more fitted animals earlier and more reliable. It will also serve as a guide in determining the most appropriate approach to be employed in the genetic improvement of the animals.

The existence of a functional relationship between multi-loci heterozygosity and fitness traits has been established in several species of animals (Coltmann *et al.*, 1998; Wu *et al.*, 2001; Jiang *et al.*, 2005; Liu *et al.*, 2006). This study was done in order to determine the correlations between the level of individual microsatellite loci heterozygosity and body weight, breast width, breast depth, keel length, and shank length among sexually matured Philippine Mallard (PM), Khaki Campbell (KC) and Pekin (PK) ducks in the Philippines.

Materials and Methods

Samples and DNA extraction

The fitness-related data and the blood samples used in determining the microsatellite loci heterozygosity were taken from a total of 90 unrelated ducks (30 each of the three genetic groups). The PK and the KC ducks were obtained from the purebred stocks that are being kept and maintained at the National Swine and Poultry Research Development Center in Tiaong, Quezon while the PM ducks were taken from a private farm in Victoria, Laguna.

The blood samples were collected by extracting approximately 1.0 ml of blood from the wing vein of each of the sample animals using a sterile disposable syringe. The collected blood was directly and immediately blotted onto Whatman® FTA® cards (Whatman International Ltd.) at an approximate rate of 0.20 ml per blot.

The recovery of genomic DNA from the blood samples was done by punching 30 discs from the blood blots in each of the Whatman® FTA® cards

using a 1.20-mm Harris® Micro-Punch (Whatman International Ltd.). The discs were then transferred into PCR tubes and washed with FTA® Purification Reagent (Whatman Inc.) until they become clear. Then a final washing was done using sterile nanopure water after which the samples were allowed to dry at room temperature before they were stored for further use. Five discs from each sample were added with 50 µl of sterile nanopure water in a PCR tube and were eluted for 10 min at 90°C using a G Storm (Gene Technologies Ltd.) thermal cycler. The eluted materials were then stored in a freezer at -20°C for future or subsequent use.

Microsatellite primers, DNA amplification and scoring

A total of 21 polymorphic duck microsatellite primers (Table 1) were used in the analysis of the individual heterozygosity levels of the sample animals. The optimization of the PCR condition for each of the primers employed in the analysis was performed using the G Storm thermal cycler.

The polymerase chain reaction (PCR) was carried out in 20.0 µl reaction volume containing 1 to 5 µl of the eluted DNA, and a final concentration of 0.50 mM each of the forward and reverse primers, 0.20 mM dNTP, 1.50 to 3.0 mM $MgCl_2$, and 0.20 to 0.30 U/µl of Taq DNA polymerase. The amplification of the microsatellites were carried out using the G Storm thermal cycler which was programmed to run for an initial denaturation of 5 minutes at 94ºC, followed by 30 cycles of 30 seconds of denaturation at 94ºC, 30 seconds of annealing at 43.8 to 59°C depending upon the primer, and 30 seconds of elongation at 72ºC. The reaction was completed with a final run at 72ºC for 5 minutes.

The DNA profiles in the PCR-amplified products were separated using 8% (w/v) polyacrylamide gels and then viewed under the Photodoc Universal Hood II (BioRad Laboratories) to visualize the bands generated from the PCR products. The bands generated were analyzed in order to determine the individual genotype of the duck samples. Genotyping involved the recording of the homozygous or heterozygous state of the ducks, as well as the determination of the size of the respective bands or alleles. Allele size was estimated by comparison with a standard ladder DNA marker (1 Kb Plus DNA Ladder).

Statistical analysis

The data analyses were facilitated with the use of the MINITAB version 14 software.

Table 1. List of the 21 duck microsatellite primers employed in the study.

No.	Name	Length, bp	Oligo sequence
1	APL2F	25	GATTCAACCTTAGCTATCAGTCTCC
	APL2R	18	CGCTCTTGGCAAATGTCC
2	APL11F	22	AACTACAGGGCACCTTATTTCC
	APL11R	22	TTGCATCAGGGTCTGTATTTTC
3	APL12F	22	AGTTGACCCTAATGTCAGCATC
	APL12R	24	AAGAGACACTGAGAAGTGCTATTG
4	APL23F	18	GAAGAGGCAGTGGCAACG
	APL23R	19	GCTGAGATGCTCCCAGGAC
5	APL26F	23	AACAGGGATAACATGAGAAGTGG
	APL26R	24	TGAGCAGCTGTCTGGTATCTATTC
6	APL36F	20	ATGCTTTGCTGTTGGAGAGC
	APL36R	19	TCCACTGGGTGCAAACAAG
7	APL83F	22	GAATAAAGTAACGGGCTTCTCT
	APL83R	19	CTGCTTGGTTTTGGAAAGT
8	APL81F	22	ATTAGAGCAGGAGTTAGGAGAC
	APL81R	19	GCAAGAAGTGGCTTTTTTC
9	APL79F	19	ACATCTTTGGCATTTTGAA
	APL79R	22	CATCCACTAGAACACAGACATT
10	APL78F	22	AACCAAGACAGAATAATCCTTA
	APL78R	19	GAACACAACTGCTTTGCTA
11	APL77F	22	TCACTTGCTCTTCACTTTCTTT
	APL77R	21	GTATGACAGCAGACACGGTAA
12	CMO12F	20	GGATGTTGCCCCACATATTT
	CMO12R	21	TTGCCTTGTTTATGAGCCATT
13	CMO11F	21	CTCCACTAGAACACAGACATT
	CMO11R	19	CATCTTTGGCATTTTGAAG
14	APH01F	22	TACCTTGCTCTTCACTTTCTTT
	APH01R	21	GTATGACAGCAGACACGGTAA
15	APH07F	19	ACATCTTTGGCATTTTGAA
	APH07R	22	CATCCACTAGAACACAGACATT
16	APH09F	20	GGATGTTGCCCCACATATTT
	APH09R	22	TTGCCTTGTTTATGAGCCATTA
17	APH10F	22	ATTAGAGCAGGAGTTAGGAGAC
	APH10R	19	GCAAGAAGTGGCTTTTTTC
18	APH11F	22	GGACCTCAGGAAAATCAGTGTA
	APH11R	19	GCAGGCAGAGCAGGAAATA
19	SMO6F	23	GGGGTGGGAAAGAAGCAGTTTAG
	SMO6R	23	TCCTGGGACTTTGAAAGTGGCTC
20	SMO7F	23	TTTTCACCCAGTTCACTTCAGCC
	SMO7R	23	GATTCAAATTTGCCGCAGGATTA
21	SMO11F	23	AAATCAACCAAAGAGGCATAGCC
	SMO11R	23	GCAGTTGTTTTGGAGGACAGACA

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Results and Discussions

Multi-loci heterozygosity

The average heterozygosity level in the 21 polymorphic microsatellites among the three genetic groups of mallard ducks is 0.3258 (Table 2). On a within breed basis, the average level of individual multi-loci heterozygosity was highest among the Pekin ducks at 0.3985 while the Khaki Campbell and Philippine Mallard ducks have average multi-loci heterozygosity levels of 0.3077 and 0.2711, respectively. Duncan's Multiple Range Test detected that the multi-loci heterozygosity of the Pekin is significantly higher ($p<0.05$) compared with that of the Khaki Campbell and the Philippine Mallard duck populations.

Table 2. Multi-loci heterozygosity of the ducks based on 21 polymorphic microsatellite markers.

Genetic groups	Multi-loci heterozygosity (Mean* ± SEM)
Philippine Mallard	0.2711 ± 0.0188 b
Khaki Campbell	0.3077 ± 0.0142 b
Pekin	0.3985 ± 0.0174 a
Over-all	0.3258 ± 0.0112

* - means with different letters are significantly different by DMRT ($p<0.05$)

Fitness characteristics

Aside from shank length that was observed to be the longest in the female PK, the male PK duck dominated the other groups in terms of bodyweight at maturity, breast depth, breast width, and keel length (Table 3). This could be substantiated by the fact that the PK is a meat type breed and is usually raised for its big body size for maximum meat yield. On the other hand, the KC and the PM are classified as egg type ducks and are raised primarily for their eggs (Bondoc, 2008; Lambio, 2010).

Table 3. Bodyweight and morphometric characteristics of the ducks at sexual maturity.

Parameters	Pekin		Khaki Campbell		Philippine Mallard	
	Male (n =15)	Female (n =15)	Male (n = 15)	Female (n =15)	Male (n = 15)	Female (n =15)
Bodyweight, kg	3.24 a	2.49 b	1.54 c	1.50 c	1.58 c	1.54 c
Breast depth, cm	10.40a	9.60 b	7.93 c	7.40 cd	6.87 d	6.80 d
Breast width, cm	15.33a	14.27 b	10.93 cd	10.67 d	11.6c	10.53 d
Keel length, cm	15.53a	13.83 b	11.07 cd	11.00 cd	11.4c	10.47 d
Shank length, cm	3.93 b	4.60 a	3.33 cd	3.07 d	3.93 b	3.80 bc

Note: In a row, means with no common letter are significantly different by DMRT ($p < 0.05$).

Heterozygosity-Fitness Correlations

On a with-in breed basis, the correlation coefficients between heterozygosity level and the fitness traits ranges from -0.252 (breast depth of the PK) to 0.267 (breast depth of PM) though no significant correlation was detected (Table 4). However, on an over-all population basis, significant positive correlations have been detected between heterozygosity and bodyweight at maturity ($r = 0.468$), breast width ($r = 0.410$), breast depth ($r = 0.443$) and keel length ($r = 0.449$). Considering the small population sizes of the three genetic groups of mallard ducks in the study, the detected significance could be explained by the principle of associative overdominance and particularly by the linkage disequilibrium model (Bierne *et al*., 2000). Either linkage disequilibrium or inbreeding alone can produce an apparent superiority of heterozygotes in a marker locus and associative overdominance arises when the effect of linkage disequilibrium on the difference between the heterozygote and the homozygote is positive (Jiang *et al*., 2005).

Table 4. Within and among population microsatellite heterozygosity and fitness correlations in the PM, KC and PK ducks.

Fitness traits	Correlation coefficients (r)			
	Philippine Mallard	Khaki Campbell	Pekin	Over-all
Bodyweight at maturity	0.262	0.112	0.070	0.468*
Breast width	0.060	0.073	-0.082	0.410*
Breast depth	0.267	0.111	-0.252	0.443*
Keel length	0.064	0.061	0.043	0.449*
Shank length	0.158	- 0.001	-0.209	0.150

* - significant Pearson's r correlation ($p<0.05$)

The significant correlation between heterozygosity and bodyweight at maturity means that, irrespective of breed, 21.9% of the variations in the bodyweight of the mallard ducks at maturity could be explained by their heterozygosity based on the 21 polymorphic microsatellite loci that were employed (Table 5). The coefficients of determination for the other traits that are significantly correlated to heterozygosity as herein detected are 20.2%, 19.6% and 16.8% for keel length, breast depth and breast width, respectively. In the native Meishan pigs, Jiang *et al.* (2005) reported that microsatellite heterozygosity level could explain 13.53% to 49.56% of the variations in the 35-day bodyweight and average daily gain of the animals.

Table 5. Result of the univariate regression analyses for fitness traits with the individual microsatellite multi-loci heterozygosity among the PM, KC and PK ducks.

Fitness trais	Microsatellite heterozygosity			
	Constant	Slope	R^2, %	P value
Bodyweight, kg.	0.9523 ± 0.22	0.0316 ± 0.01	21.9	0.000
Breast width, cm.	9.5516 ± 0.67	0.0823 ± 0.02	16.8	0.000
Breast depth, cm.	6.0740 ± 0.47	0.0642 ± 0.01	19.6	0.000
Keel length, cm.	9.4366 ± 0.62	0.0853 ± 0.02	20.2	0.000
Shank length, cm.	3.4664 ± 0.23	0.0095 ± 0.01	2.3	0.158

The detection of significant correlation between the individual microsatellite multi-loci heterozygosity and the selected fitness traits namely

bodyweight at maturity, breast width, breast depth and keel length, suggests that these traits could be altered by manipulating the degree of the individual heterozygosity of the ducks in the 21 polymorphic microsatellite markers involved. In their investigation, Liu *et al.* (2006) observed that positive correlation exists between microsatellite heterozygosity and the bodyweight of a Chinese native chicken. According to them, the bodyweight of the Chinese native chicken could be improved by increasing microsatellite heterozygosity.

The significance of the correlations between the individual microsatellite multi-loci heterozygosity and the selected fitness traits at the population level hints that a more significant effect in the improvement of these traits, e.g. increasing the bodyweight potential at maturity, could be realized by employing selection of highly heterozygous individuals as parental or breeder stocks. Individuals with high degree of microsatellite loci heterozygosity are expected to have higher bodyweights or bigger body size and therefore are expected to produce offsprings with high bodyweight potentials. This is in agreement with the basic animal breeding principle stating that traits which are moderate to highly heritable are best improved through phenotypic selection (Bourdon, 1997). The bodyweight of the PM duck at 40 weeks of age is highly heritable (Deeden, 2005) suggesting that it is governed by the action of additive genes and can be best improved through selection.

In addition, the individual performance potential of the three genetic groups of mallard duck in terms of bodyweight, breast width, breast depth, and keel length at maturity could be ascertained early in their life by employing linear regression analysis on the degree of their individual heterozygosity on the 21 polymorphic microsatellite loci. The regression coefficient values (Table 5) indicate that for every percentage increase in the heterozygosity level of the mallard ducks, a corresponding increase of 0.0316 kg in bodyweight, 0.0823 cm in breast width, 0.0642 cm in breast depth, and 0.0853 cm in keel length will be realized.

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