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MICROSATELLITE-BASED GENETIC DIVERSITY AND RELATIONSHIP ANALYSES OF THREE GENETIC GROUPS OF DOMESTICATED MALLARD DUCKS (*Anas platyrhynchos domesticus* L.)

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ABSTRACT

The genetic diversity and relationships among the Philippine Mallard, Khaki Campbell and Pekin ducks were analyzed by employing 28 microsatellite primers which were amplified from the genome of 30 animals representing each genetic group. Twenty-one of the 28 microsatellite primers employed are polymorphic. The average observed and effective number of allele ranges from 2.238 to 2.714 and 1.565 to 1.934, respectively, while the average observed and expected heterozygosity ranges from 0.297 to 0.432 and 0.308 to 0.422, respectively. The within population inbreeding estimate among the three genetic groups of mallard ducks is -0.0020 while the total inbreeding estimate is 0.1292 suggesting that random genetic drift could be happening in the duck populations considered. On the other hand, the measurement of population differentiation has a value of 0.1309. Relationship analyses reveal that the Philippine Mallard is genetically closer to the Khaki Campbell (0.0944) than with the Pekin (0.1523). The genetic distance between the Khaki Campbell and the Pekin is 0.1386.

Keywords: genetic diversity, genetic relationship, microsatellite, Philippine mallard duck

INTRODUCTION

Duck raising in the Philippines ranks next to the chicken industry in economic importance as source of egg and poultry meat (Lambio, 2009a; PCARRD, 2010). It plays a very important role in the nutritional improvement and in augmenting the income of rural families through the production of high-priced protein products out of locally available feed resources (PCARRD, 2005). The local duck industry, however, is being faced by a lot of problems which include the lack of quality breeder stocks (Lambio, 2009b).

Preventing the genetic deterioration and the development of highly productive local ducks could be done by improving their genetic make-up. In determining what genetic resources should be used in designing a sustainable breeding and conservation programs, there is a need to understand the genetic diversity and relationships among the three major duck genetic groups in the country: the Philippine Mallard (PM), Khaki Campbell (KC), and the Pekin (PK).

The genetic diversity within and between populations can be determined at the morphological, biochemical and molecular levels. However, genetic variation analysis based on morphological and biochemical markers has limited power for genetic and bio-diversity studies (FAO, 2007; Hanotte and Jianlin, 2005). Genetic characterization based on molecular markers offers greater power of detection than do phenotypic methods (de Vicente *et al*., 2005). In livestock genetic characterization studies, the most popular markers are the microsatellites (Sunnucks, 2000; Sukla *et al*., 2006). Also known as simple sequence repeats or short tandem repeats, microsatellites are tandemly repeated

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motifs of one to six nucleotides which are found in both coding and non-coding regions of all prokaryotic and eukaryotic genomes (Hsiao *et al*., 2008; Huang *et al*., 2006; Tu *et al*, 2006).

Because of their high polymorphism, abundant distribution throughout the genome, co-dominant nature, high reproducibility, relative ease of scoring, and easy and quick detection, the microsatellites are the markers of choice for genetic diversity studies (Gholizadeh and Mianji, 2007; Duran *et al.*, 2009). The genetic diversity and relationships among the major Philippine duck genetic groups based on microsatellite polymorphisms have not been established hence this study.

MATERIALS AND METHODS

Blood samples were collected from 90 unrelated ducks that include 30 each of the PM, KC and PK. The KC and PK ducks came from the National Swine and Poultry Research and Development Center in Tiaong, Quezon while the PM ducks were sourced from a private farm in Victoria, Laguna. Approximately one mL of blood was collected from the wing vein of each of the ducks using a sterile disposable syringe. The collected blood was immediately blotted onto a Whatman® FTA® card (Whatman International Ltd.).

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 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 in the G Storm (Gene Technologies Ltd.) thermal cycler. The eluted materials were then stored in a freezer at -20ºC for future or subsequent use.

The study made use of the 28 duck microsatellite primers that are shown in Table 1. The same set of primers was employed by Li *et al*. (2006) in the evaluation of the molecular genetic diversity of native duck breeds in China. Khan Ahmadi *et al*. (2007) also used 12 of these primers to analyze the genetic diversity of the Pekin and the Muscovy ducks in northern Iran.

The optimization of the PCR condition for each of the primers was performed using the G Storm thermal cycler. The 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 Taq DNA polymerase. In the amplification of the microsatellites, the thermal cycler 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 59ºC 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% PAGE and then viewed under the Photodoc Universal Hood II (BioRad Laboratories) to visualize the bands generated from the PCR products. Genotyping was done by analyzing the bands and recording 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) using the Gel Doc XR of the Quantity One version 4.6.3 software (BioRad Laboratories, USA).

The POPGENE version 1.32 was employed in the determination of the observed (N_o) and effective number of alleles (N_e), observed (H_o) and expected heterozygosity (H_e), fixation or heterozygosity deficiency index (F_{is}), within population inbreeding estimate

Table 1. Primer sequences used for the amplification of 28 microsatellite loci in the Khaki Campbell, Philippine Mallard and Pekin duck populations.

Loci	Sequences (5' - 3')	Loci	Sequences (5' - 3')
APL2F	GATTCAACCTTAGCTATCAGTCTCC	CMO11F	CTCCACTAGAACACAGACATT
APL2R	CGCTCTTGGCAAATGTCC	CMO11R	CATCTTTGGCATTTTGAAG
APL11F	AACTACAGGGCACCTTATTTCC	APH01F	TACCTTGCTCTTCACTTTCTTT
APL11R	TTGCATCAGGGTCTGTATTTTC	APH01R	GTATGACAGCAGACACGGTAA
APL12F	AGTTGACCCTAATGTCAGCATC	APH07F	ACATCTTTGGCATTTTGAA
APL12R	AAGAGACACTGAGAAGTGCTATTG	APH07R	CATCCACTAGAACACAGACATT
APL23F	GAAGAGGCAGTGGCAACG	APH09F	GGATGTTGCCCCACATATTT
APL23R	GCTGAGATGCTCCCAGGAC	APH09R	TTGCCTTGTTTATGAGCCATTA
APL26F	AACAGGGATAACATGAGAAGTGG	APH10F	ATTAGAGCAGGAGTTAGGAGAC
APL26R	TGAGCAGCTGTCTGGTATCTATTC	APH10R	GCAAGAAGTGGCTTTTTTC
APL36F	ATGCTTTGCTGTTGGAGAGC	APH11F	GGACCTCAGGAAAATCAGTGTA
APL36R	TCCACTGGGTGCAAACAAG	APH11R	GCAGGCAGAGCAGGAAATA
APL83F	GAATAAAGTAACGGGCTTCTCT	APH14F	GAATAAAGTAACGGGCTTCTCT
APL83R	CTGCTTGGTTTTGGAAAGT	APH14R	CTGCTTGGTTTTGGAAAGT
APL82F	GGACCTCAGGAAAATCAGTGTA	SMO6F	GGGGTGGGAAAGAAGCAGTTTAG
APL82R	GCAGGCAGAGCAGGAAATA	SMO6R	TCCTGGGACTTTGAAAGTGGCTC
APL81F	ATTAGAGCAGGAGTTAGGAGAC	SMO7F	TTTTCACCCAGTTCACTTCAGCC
APL81R	GCAAGAAGTGGCTTTTTTC	SMO7R	GATTCAAATTTGCCGCAGGATTA
APL80F	GGATGTTGCCCCACATATTT	SMO9F	TTTGGAGTTTGGAGTTCGTGGGG
APL80R	TTGCCTTGTTTATGAGCCATTA	SMO9R	ATTTCCCTGCAAAACTTACGGCA
APL79F	ACATCTTTGGCATTTTGAA	SMO10F	TCCTAGCGACAGCAATTCTAATG
APL79R	CATCCACTAGAACACAGACATT	SMO10R	CATTGTTCATTGTTTCTTCTTCA
APL78F	AACCAAGACAGAATAATCCTTA	SMO11F	AAATCAACCAAAGAGGCATAGCC
APL78R	GAACACAACTGCTTTGCTA	SMO11R	GCAGTTGTTTTGGAGGACAGACA
APL77F	TCACTTGCTCTTCACTTTCTTT	SMO12F	CCTGGTGGGATAGGTTTAAAATG
APL77R	GTATGACAGCAGACACGGTAA	SMO12R	TGTTCATCAAAAGCAGAGAGGGG
CMO12F	GGATGTTGCCCCACATATTT	SMO13F	ACCATCTTCCTTTCCTCCCAACC
CMO12R	TTGCCTTGTTTATGAGCCATT	SMO13R	GGGCTTGAGGCATACACTCCCTA

(F_{IS}), total inbreeding estimate (F_{IT}), measurement of population differentiation (F_{ST}), Nei's genetic distance and genetic identity measures as well as in the Hardy-Weinberg equilibrium test for the loci employed. The software was also used in the generation of the dendrogram of the relationships among the three genetic groups of mallard ducks studied.

The time of divergence was estimated by using the formula D = 2αt (Sharma *et al*.,2009) where D is the value of Nei's standard genetic distance, α is the mutation rate assumed at 1.4 x 10^{-4}locus^{-1}gamete^{-1} and t is the time of divergence in terms of number of generations.

RESULTS AND DISCUSSION

All of the 28 microsatellite primers were amplified. However, only 21 were found to be polymorphic. Monomorphism was detected among the genomes of the mallard ducks in the APL 82, APL 80, APH 14, SMO 9, SMO 10, SMO 12 and SMO 13. Khan Ahmadi *et al*. (2007) were not able to amplify the primers SMO1 and SMO12 and at the same time detected monomorphism among the genome of Pekin ducks in northern Iran in the SMO7 and SMO8. The Hardy-Weinberg equilibrium tests showed that none of the 28 microsatellite sites were at equilibrium.

The observed and the expected number of alleles per locus ranges from one to five and one to 3.358, respectively (Table 2). The highest N_o was observed in the APL 23 of PK while the highest N_e was detected from the APL 23 of the KC. The KC was found out to be the most genetically diversified based on the average N_o and N_e per locus while the least diversified is the PM. In their study on the PM, Dungca (2009) and Paulmanal (2010) reported lower N_o and N_e per locus which could be attributed to the smaller sample sizes and fewer primers that they have employed. Li *et al*. (2006) reported higher N_o and N_e per

Table 2. Allele size range, observed (N_o) and effective (N_e) number of alleles per microsatellite locus in the Philippine Mallard, Khaki Campbell and Pekin duck genomes.

Loci	Allele size range, bp	Philippine Mallard		Khaki Campbell		Pekin	
		N_o	N_e	N_o	N_e	N_o	N_e
APL 2	112-129	4.000	2.270	4.000	2.708	4.000	2.699
APL 11	88-131	4.000	1.791	3.000	1.692	4.000	2.146
APL 12	126-165	4.000	1.274	3.000	1.114	3.000	1.675
APL 23	146-239	3.000	2.663	4.000	3.358	5.000	3.306
APL 36	145-233	3.000	2.583	4.000	2.145	4.000	3.004
APL 26	150-163	2.000	1.946	3.000	2.082	2.000	1.210
APL 83	100-134	2.000	1.514	3.000	2.867	4.000	1.865
APL 81	148-154	1.000	1.000	2.000	1.461	2.000	1.401
APL 79	234-264	1.000	1.000	2.000	1.508	1.000	1.000
APL 78	220-338	2.000	1.684	2.000	1.415	2.000	1.352
APL 77	200-267	2.000	1.220	2.000	1.153	1.000	1.000
CMO 12	96-130	2.000	1.514	4.000	3.130	4.000	1.840
CMO 11	244-267	1.000	1.000	2.000	1.774	1.000	1.000
APH 01	197-260	2.000	1.220	2.000	1.153	1.000	1.000
APH 07	220-280	1.000	1.000	3.000	1.764	3.000	1.745
APH 09	100-139	2.000	1.514	3.000	2.867	4.000	1.865
APH 10	146-155	2.000	1.342	2.000	1.645	2.000	1.401
APH 11	172-193	2.000	1.684	1.000	1.000	2.000	2.000
SMO 6	118-136	2.000	1.835	3.000	2.667	3.000	2.667
SMO 7	187-245	3.000	1.681	3.000	1.612	3.000	2.217
SMO 11	187-209	2.000	1.142	2.000	1.508	1.000	1.000
Mean		2.238	1.565	2.714	1.934	2.667	1.733

locus among the native duck breeds in China while Khan Ahmadi *et al*. (2007) observed an average of 2.2 alleles among the genomes of PK ducks in northern Iran.

The proportions of observed and expected heterozygosity in a given locus range from zero to one and zero to 0.715, respectively (Table 3). The average H_o was highest in the PK and the highest average H_e was observed in the KC population. The lowest average H_o and H_e were observed in the PM ducks. In the PM, Dungca (2009) and Paulmanal (2010) reported higher levels of observed heterozygosity at 0.35 and 0.42, respectively, but lower expected heterozygosity at 0.33. The native ducks in China have a heterozygosity level that ranges from 0.514 to 0.617 (Li *et al*., 2006) while Khan Ahmadi *et al*. (2007) observed an average of 0.82 heterozygosity level among the Pekin ducks of northern Iran.

There is a heterozygote deficiency in the populations of KC (20.98%) and the PM (3.22%) while there is an excess (22.62%) in the PK (Table 4). Numerous factors such as inbreeding, locus under selection or genetic hitchhiking, presence of null or non-amplifying alleles, and presence of population substructure or Wahlund effect may be responsible for lack of heterozygotes in a population (Nei, 1987; Khan Ahmadi *et al*., 2007).

Table 3. Observed (H_o) and expected (H_e) heterozygosity per microsatellite locus in the Philippine Mallard, Khaki Campbell, and Pekin duck genomes.

Loci	Philippine Mallard		Khaki Campbell		Pekin	
	H_o	H_e	H_o	H_e	H_o	H_e
APL 2	0.400	0.569	0.786	0.642	1.000	0.642
APL 11	0.467	0.449	0.107	0.416	0.539	0.545
APL 12	0.167	0.219	0.107	0.105	0.539	0.411
APL 23	0.467	0.635	0.321	0.715	0.808	0.711
APL 36	0.567	0.623	0.857	0.544	0.923	0.680
APL 26	0.300	0.494	0.286	0.529	0.039	0.177
APL 83	0.367	0.345	0.571	0.663	0.577	0.473
APL 81	0.000	0.000	0.393	0.321	0.346	0.292
APL 79	0.000	0.000	0.000	0.343	0.000	0.000
APL 78	0.567	0.413	0.357	0.299	0.308	0.266
APL 77	0.200	0.183	0.143	0.135	0.000	0.000
CMO 12	0.367	0.345	0.536	0.693	0.577	0.465
CMO 11	0.000	0.000	0.000	0.444	0.000	0.000
APH 01	0.200	0.183	0.143	0.135	0.000	0.000
APH 07	0.000	0.000	0.036	0.441	0.539	0.435
APH 09	0.367	0.345	0.571	0.663	0.577	0.473
APH 10	0.300	0.259	0.536	0.399	0.346	0.292
APH 11	0.567	0.413	0.000	0.000	1.000	0.510
SMO 6	0.433	0.463	0.643	0.636	0.769	0.637
SMO 7	0.500	0.412	0.464	0.386	0.192	0.182
SMO 11	0.000	0.127	0.000	0.343	0.000	0.000
Mean	0.297	0.308	0.327	0.422	0.432	0.342

The observed within population inbreeding estimate across the three genetic groups is -0.0020 (Table 5) indicating that random mating is happening in the mallard duck populations considered. Moreover, the mean total inbreeding estimate suggests that the entire population had 12.92% deficit of heterozygotes. These imply that, in the absence of inbreeding, the Wahlund effect as a result of random genetic drift could be responsible for the lack of heterozygotes in the KC and PM populations. Carpena *et al.* (1993) stated that random mating in a small population will lead into increased homozygosity as a result of random genetic drift.

On the other hand, the measurement of population differentiation reveals a considerable genetic differentiation among the mallard duck breeds. Approximately 13.09% of the total genetic variation among them was due to breed differences and the remaining 86.91% was due to differences among individuals within breeds. Although a little bit lower, this genetic variation level is comparable to the 14.4% detected by Su and Chen (2009) among four breeds of Chinese indigenous laying-type ducks.

Table 4. Within population heterozygosity estimate (F_{is}) per locus in the Philippine Mallard, Khaki Campbell and Pekin duck populations.

Loci	Philippine Mallard	Khaki Campbell	Pekin
APL 2	0.2850	-0.2457	-0.5887
APL 11	-0.0566	0.7379	-0.0083
APL 12	0.2248	-0.0435	-0.3358
APL 23	0.2527	0.5422	-0.1580
APL 36	0.0752	-0.6057	-0.3836
APL 26	0.3829	0.4503	0.7787
APL 83	-0.0802	0.1224	-0.2440
APL 81	*	-0.0244	-0.2093
APL 79	*	1.0000	*
APL 78	-0.3953	-0.2174	-0.1818
APL 77	-0.1111	-0.0769	*
CMO 12	-0.0802	0.2127	-0.2642
CMO 11	*	1.0000	*
APH 01	-0.1111	-0.0769	*
APH 07	*	0.9175	-0.2617
APH 09	-0.0802	0.1224	-0.2440
APH 10	-0.1765	-0.3659	-0.2093
APH 11	-0.3953	*	-1.0000
SMO 6	0.0476	-0.0286	-0.2308
SMO 7	-0.2346	-0.2235	-0.0788
SMO 11	1.0000	1.0000	*
Mean	0.0322	0.2098	-0.2262

* - all samples were homozygous.

Table 5. Summary of the F-statistics per locus in the PM, KC and PK duck genomes.

Loci	Within population inbreeding estimate (F_{IS})	Total inbreeding estimate (F_{IT})	Population differentiation estimate (F_{ST})
APL 2	-0.2012	-0.0548	0.1218
APL 11	0.1966	0.3914	0.2425
APL 12	-0.1269	-0.0438	0.0737
APL 23	0.2116	0.2271	0.0197
APL 36	-0.2939	-0.2264	0.0522
APL 26	0.4709	0.5888	0.2229
APL 83	-0.0417	0.2057	0.2375
APL 81	-0.2277	-0.1405	0.0711
APL 79	1.0000	1.0000	0.1538
APL 78	-0.2830	-0.2583	0.0193
APL 77	-0.0966	-0.0606	0.0328
CMO 12	-0.0020	0.2170	0.2185
CMO 11	1.0000	1.0000	0.2400
APH 01	-0.0966	-0.0606	0.0328
APH 07	0.3322	0.4030	0.1060
APH 09	-0.0417	0.2057	0.2375
APH 10	-0.2661	-0.2453	0.0164
APH 11	-0.7290	-0.3534	0.2172
SMO 6	-0.0824	-0.0457	0.0339
SMO 7	-0.2014	-0.1740	0.0228
SMO 11	1.0000	1.0000	0.0945
Mean	-0.0020	0.1292	0.1309

Based on the Nei's original measure of genetic distance, the PM and KC are more closely related to each other than either is to the PK. A similar pattern was observed in the genetic distances among the three genetic groups in terms of the unbiased genetic distance measure (Table 6).

Based on the original Nei's measure (Nei, 1972), the genetic identity estimate between the PM and the KC was highest at 0.9099 followed by that between the KC and PK and between the PM and PK with 0.8706 and 0.8588, respectively (Table 6). The unbiased measures (Nei, 1978), likewise, show a similar pattern of genetic identity among the groups.

The dendrogram (Figure 1) constructed indicates that the Khaki Campbell and the Philippine Mallard are more closely related to each other and they are clustered differently from the Pekin. This substantiated the fact that the Khaki Campbell and the Philippine Mallard are both of the egg-type while the Pekin is of the meat-type.

The estimated time of divergence among the genetic groups is shortest between the Khaki Campbell and Philippine Mallard at 674 generations. This is followed by the time of divergence between the Khaki Campbell and Pekin at 990 generations and that of the Philippine Mallard and Pekin at 1088 generations (Table 7).

Table 6. Nei's original and unbiased measures of genetic distance and identity estimates among the Philippine Mallard, Khaki Campbell and Pekin ducks.

Breed grouping	Nei's genetic distance measure estimates		Nei's genetic identity measure estimates	
	Original	Unbiased	Original	Unbiased
PM and KC	0.0944	0.0894	0.9099	0.9145
PM and PK	0.1523	0.1480	0.8588	0.8625
KC and PK	0.1386	0.1330	0.8706	0.8755

Table 7. Estimated time of divergence among the Philippine Mallard, Khaki Campbell and Pekin based on the 21 microsatellite loci.

Breed grouping	Nei's standard genetic distance (D_S)	Divergence time (no. of generations)
PM and KC	0.0944	674
PM and PK	0.1523	1088
KC and PK	0.1386	990

Considering a generation interval of one year (Bondoc, 2009), the current findings imply that the lineages of the three genetic groups started to separate 1088 years ago between the Philippine Mallard and the Pekin, 990 years between the Khaki Campbell and Pekin, and 674 years between the Philippine Mallard and the Khaki Campbell.

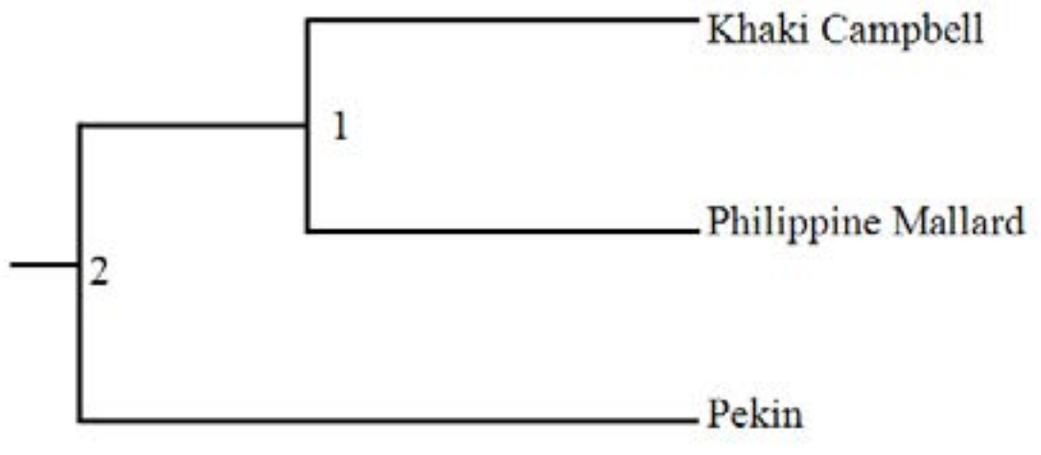

Figure 1. Denogram showing relativity of Khaki Campbell and Philippine Mallard duck in relation to Pekin.

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