(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

MICROSATELLITE BASED GENETIC CHARACTERISATION OF INDIAN MUSCOVY DUCK (*CAIRINA MOSCHATA*)

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ABSTRACT

No information is available on genetic characteristics of Indian Muscovy duck. Blood samples were collected from 52 Muscovy ducks from Manipur, Assam and West Bengal. DNA was isolated and data generated on 24 microsatellite loci. Population demographic estimations were made using various qualitative as well as quantitative tests of severe reduction in effective population size and population expansions. There has been no severe reduction in the population of Muscovy ducks as revealed by genetic bottleneck studies. The population of Cairina moschata has undergone expansions as revealed by k and g tests. The gene diversity of Indian domestic ducks is quite high with mean number of alleles per locus as 11.46. The mean effective number of alleles was 5.02. The mean heterozygosity was found to be 0.54 while the expected heterozygosity was 0.72. This along with the statistically significant F_{IS} value (0.25), point towards the existence of population structure due to local inbreeding in Muscovy duck population. The Muscovy ducks in India need to be treated separately from Anas platyrhyncos and separate breeding and conservation strategies shall be needed.

Keywords: Carina duck, microsatellite, Muscovy ducks, bottleneck.

INTRODUCTION

The Muscovy duck also commonly known as warty duck is native to Latin America. It is one of the greater wood ducks belonging to genus *Cairina* included in the tribe Cairinini belonging to subfamily Anatine of family Anatidae. The Muscovy duck distinguishes itself most sharply from the common domestic duck as there is an area of bare skin from the bill to just above and behind to the eyes which is covered in what superficially resemble warty outgrowths or caruncles. The caruncles in males culminate in large red fleshly knob at the base of the bill between the nostrils. This feature becomes more conspicuous during the breeding reason. The Muscovy differs from the common duck in that the male is very much larger that the female and is more aggressive. The carcass of Muscovy duck is considered to be superior to table duck due to more lean meat and less fat however the growth of Muscovy duck is very slow compared to table duck and hence not

economical. Muscovy has never been exposed to sophisticated breeding programs (Clayton 1984) but gives 12-14 eggs in a clutch. The egg size ranges from 65 to 85 g with incubation period of 35 days (one week longer then the table duck, *Anas platyrhyncos*). The exact distribution and number of Muscovy ducks are not known in the country. In India, Muscovy ducks are found almost at all the geographical locations where the ducks are found. The Muscovy is found in Manipur, Assam, West Bengal and Orissa. There is preponderance of Muscovy ducks in Manipur with its number decreasing as we move to Assam and later to West Bengal and Orissa.

Since no information is available for the Muscovy ducks of India. The present study was undertaken for characterization of Muscovy duck using microsatellite markers and estimation of population demographic features. The inbreeding and heterozygosity level of these ducks was also estimated.

MATERIALS AND METHODS

Blood samples of 48 Muscovy ducks were collected from Manipur, 2 each from Assam and West Bengal. Manipur state has most of the ducks as Muscovy compared to the table duck (*Anas platyrhyncos*). The animals were selected at random ensuring that as far as possible these were unrelated and the samples represented Manipuri Muscovy ducks. 1 to 2 ml of whole blood was collected from wing vein in EDTA coated vacutainer tubes and transported to laboratory at 0-5^{0}C. The DNA was extracted from whole blood using the standard protocol (Sambrook *et al*. 2001). The concentration of DNA was adjudged using comparison with the standard DNA marker concentration on agarose gel. The quality of DNA was checked on 0.8% agarose gel prepared in TE buffer. A total of 24 heterologus microsatellite loci were chosen for the study. These microsatellites originally belonged to the species *Anas platyrhyncos* but showed amplification in Muscovy ducks. The PCR conditions were standardized for all of the 24 primer pairs selected for the study. PCR amplification was carried out in 20 µl reaction containing 50 ng genomic DNA, 150 µM dNTP, 4 pmol of forward (labeled) and reverse primers, 1 U Taq DNA polymerase and 1 X reaction buffer (containing 1.5 mM $MgCl_2$). Amplification was carried out in ABI 9700 with initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30s, annealing temperature for 45s and extension at 72°C for 45s. The final cycle was followed by an extension step at 72°C for 10 min. The PCR products were visualized on 2% agarose gel using 1 X TAE buffer containing 200ng/ml of Ethidium bromide. The genotyping was carried out on ABI - 3100 AVANT automated DNA Sequencer with LIZ 500 (Applied Biosystems) as internal lane standard (size standard). The post PCR multiplexing was used to simultaneously genotype 3 or 4 loci depending upon the size and dye label of the PCR product. The sizing and allele calling was performed using Genotyper ver. 4.0 software (Applied Biosystems). The allele data thus generated was used for further statistical analysis.

The allelic frequencies were estimated and have been depicted locus wise in Fig. 1. The heterozygosity, gene diversity and the polymorphic information content were estimated using

POPGENE software (Yeh *et al.* 1999). A closely related diversity measure is the polymorphism information content (PIC) (Botstein *et al.* 1980). The allele sharing distance commonly called the shared allele distance D_{SA} (Chakraborty and Jin 1993) was estimated using the software Microsatellite Analyser 4.05 (Dieringer and Schlötterer 2003). The Correspondence Analysis was carried out using GENETIX software (Belkhir K *et al*. 1996-2004). The Hardy Weinberg exact test was performed using GENEPOP software (Raymond and Rousset, 1995). The parameters set up for the exact test were the alternative hypothesis H_1 being Heterozygotic deficiency, 10,000 dememorisation steps with 100 batches each having 500 iterations. The F_{IS} values were estimated for each of the 24 loci for (Weir and Cockerham 1984 and Robertson and Hill 1984) F statistics.

We utilized three quantitative tests for each population to know if they were in mutation drift equilibrium. The tests applied were Sign rank test, Standardised differences test and Wilcoxon rank test as implemented in the software Bottleneck (Piry *et al.* 1999). We utilized all the three models of microsatellite evolution viz; Infinite allele Model (IAM), Two Phase Model (TPM) and Stepwise Mutation Model (SMM). The Neighbor Joining tree based on the interindividual genetic distances based on allele sharing was constructed using algorithm of Saitou and Nei (1987) as implemented in Phylip software (Felsenstein *et al*. 1993).

The *k*-test of Reich and Goldstein (1998) which exploits differences between the expected distributions of alleles in populations at Mutation Drift Equilibrium and populations that have recently expanded. The *g*-test of Reich and Goldstein (1998) which compares the between-loci variance in the number of repeats with a theoretical expectation derived assuming that the loci follow SMM and that the population size is stable. We performed both the *k*- and the *g*-tests. *k*-statistics were calculated for each locus, and the significance of the proportion of positive *k* values was based on a binomial distribution with the probability of a positive *k* set conservatively as 0.515 (Reich *et al.* 1999). Significance levels for the *g*-test were compared to the values given in Reich *et al.* (1999).

RESULTS AND DISCUSSION

The locus name, number of alleles and allele size ranges are given in Table 1. The total number of alleles found at 24 microsatellite loci to be 275 with a mean number of alleles to be 11.46. However most of the alleles were found to be at low frequency resulting in severe reduction in the effective number of alleles to 5.0185. The number of alleles with a frequency of greater than 5% was 4.58. The heterozygosity level of ducks at each locus is given in Table 1. The mean heterozygosity was found to be 0.5401. The expected heterozygosity was 0.7250. The large value of expected heterozygosity can be attributed to large number of allele found in most of the loci used for the study. The loci selected for the study were however heterologus as these loci have been derived from *Anas platyrhyncos* but could be successfully amplified in *Cairina moschata*. The observed heterozygosity was less than the expected heterozygosity and this can be attributed to local

inbreeding effects. The overall F_{IS} value was found to be 0.2479, which was significantly different from zero and points to existence of local population structure. The F_{IS} values were positive for 20 loci out of a total of 24 loci studied. The exact test of population differentiation as implemented in GENEPOP software revealed similar results with Weir and Cockerham (1984) estimates in which the null hypothesis of heterozygotic excess was rejected in favor of alternative hypothesis of heterozygote deficiency. Similarly in the Robertson and Hill (1984) estimates 21 loci had positive values signifying heterozygotic deficiency. This also approved of local inbreeding effects of the Muscovy ducks. The Ewens-Watterson test of neutrality revealed that all the loci selected were neutral in character as all the observed values were between the L95 and U95 values obtained from 1000 simulations.

Table 1. Number of alleles (observed N_a, effective N_e), allele size range, F statistics, and Heterozygosity Statistics for each of 24 microsatellite loci.

Locus	No. of alleles (Na)	Effective no. of alleles (Ne)	Size range	Observed Heterozygosity	Expected Heterozygosity	F_{IS}
APH01	6	1.56	189-201	0.31	0.36	0.14
Caud 01	9	6.04	277-303	0.73	0.84	0.12
Caud 10	10	5.18	116-136	0.42	0.81	0.48
Caud 17	14	5.18	192-248	0.44	0.81	0.45
Caud 13	12	6.53	086-120	0.77	0.86	0.09
Caud 23	7	3.94	161-163	0.52	0.75	0.30
Caud 25	8	3.51	224-292	0.38	0.72	0.46
Caud 33	5	2.02	198-206	0.27	0.51	0.47
APH 10	11	5.55	111-149	0.98	0.83	-0.20
Caud 16	11	2.21	188-216	0.60	0.55	-0.09
Caud 19	26	14.98	086-206	0.87	0.94	0.07
Caud 31	12	6.33	110-142	0.46	0.85	0.45
APH 09	8	2.91	086-116	0.46	0.66	0.30
Caud 26	4	1.60	139-147	0.31	0.38	0.18
Caud 24	25	9.18	203-339	0.25	0.90	0.72
Caud 35	12	8.84	186-232	0.71	0.90	0.20
APH 03	7	1.44	183-217	0.21	0.31	0.31
APH 07	10	3.98	203-261	0.42	0.76	0.44
Caud 22	11	3.45	099-141	0.94	0.72	-0.33
Caud 27	13	4.93	088-118	0.62	0.81	0.23
Caud 04	10	2.45	186-222	0.44	0.60	0.25

Caud 11	13	7.22	120-150	0.31	0.87	0.64
Caud 32	14	6.48	102-134	0.69	0.85	0.18
MCW 328	10	4.92	193-223	0.85	0.80	-0.06
Mean	**11.46±1.11**	**5.02 ±0.62**		**0.54±0.02**	**0.73±0.02**	**0.25**

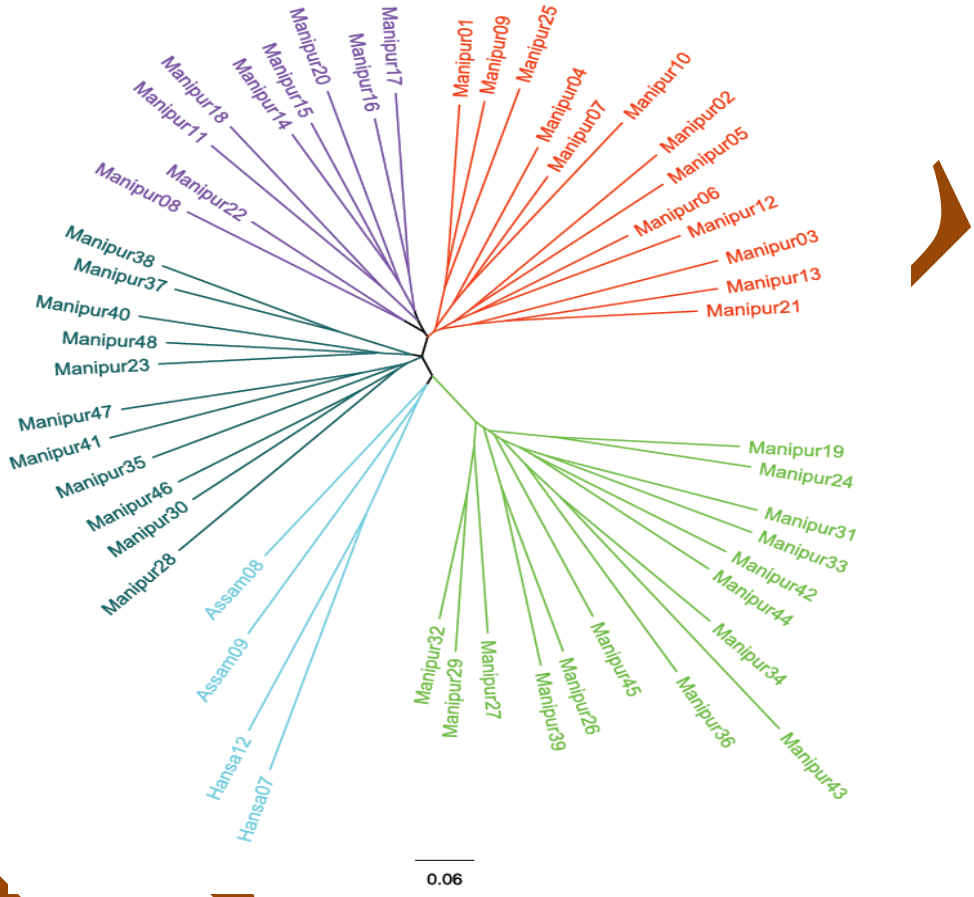


Fig. 1. The Neighbor Joining tree showing four distinctive clusters of Muscovy ducks.

The inter individual distances D calculated on the basis of allele sharing method (D_{SA}) and Neighbor Joining tree was constructed as shown in Fig. 2. The birds of Assam and West Bengal were separated from rest of the birds of Manipur. This can be attributed to the different genotypes of the birds of Manipur and Assam as they belong to different geographical regions. The Manipur birds formed 4 distinctive sub-clusters with two major clusters.

In *Cairina moschata*, the sample size was 52 i.e., 104 haploid genomes. Out of 24 loci the expected number of loci with heterozygote excess was 14.40 under IAM, 14.26 under TPM and 14.15 under SMM. The numbers of loci with heterozygosity excess observed in the study were 16, 9 and 1 loci respectively for the three mutational models. The probability values were 0.3283 (IAM), 0.0249 for (TPM) and 0.0000 for (SMM) respectively. The sign test reveals Muscovy duck population to be

under mutation drift equilibrium under IAM and TPM while the null hypothesis of mutation drift equilibrium is rejected for SMM. Similar results were obtained for the standardized difference test in which the T2 values were calculated and compared to table value. The null hypothesis of mutation drift equilibrium is accepted for IAM which had T2 value of 0.358 (<1.645) while these values were negative for TPM (-4.721) and SMM (-13.915). In both TPM as well SMM, there was significant heterozygotic deficiency instead of heterozygote excess. Similarly the Wilcoxon test for *Cairina moschata* also reveals similar results the probability values being 0.00000 (<0.05) rejecting the null hypothesis of mutation drift equilibrium being a case of heterozygotic deficiency.

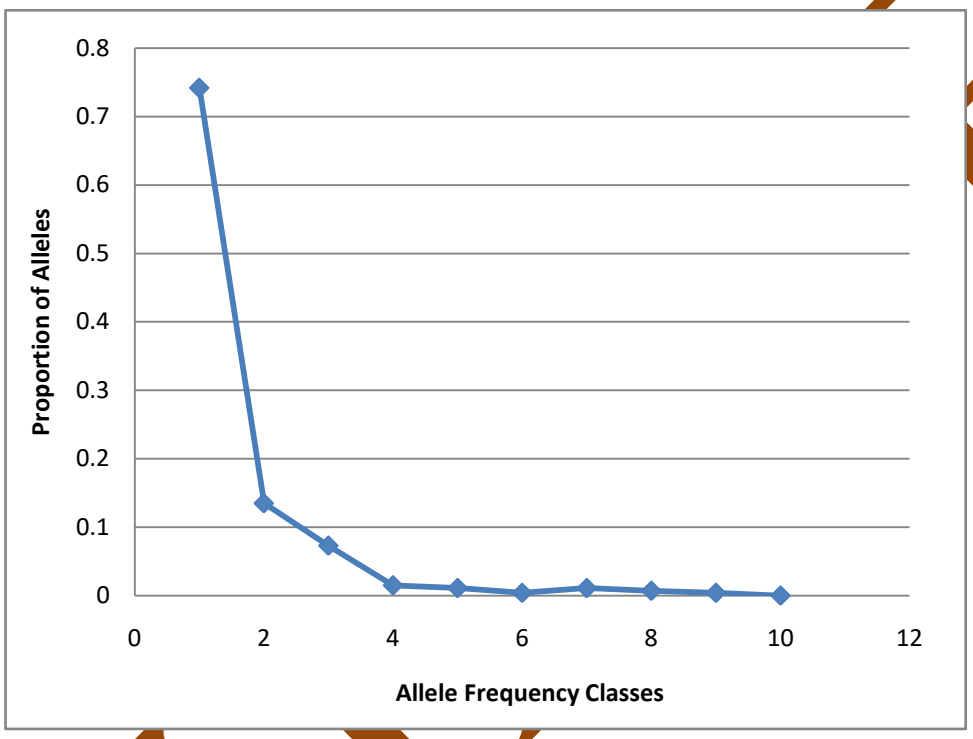


Fig. 2. Normal L shaped curve of *Cairina moschata* indicating no recent bottleneck or reduction in effective population size.

The mode shift reveals no distortion of allelic frequencies distribution and the graphic representation is normal L-shaped distribution (Fig. 3). Thus there was no bottleneck in the Muscovy duck of Manipur and hence the population has not undergone severe reduction in effective population size.

The inter-locus test (g test) was conducted and the value was found to be 3.126. This was higher than the fifth percentile cut off of g value (Reich *et al*. 1999). The within locus test (k test) showed 15 loci to be with negative values while 9 loci had positive values. The number of loci with negative value were thus significantly greater than the number of loci with the positive value and thus the null hypothesis of constancy of population size of Muscovy duck was rejected (P=0.12127).

The factorial analysis revealed that the 4 factors contribute 6.85, 6.23, 4.91 and 4.191 to the total variation. The Correspondence Analysis revealed Assam ducks and West Bengal ducks to be distinctive from Manipur ducks. The Manipuri ducks formed two distinctive clusters (Fig. 4).

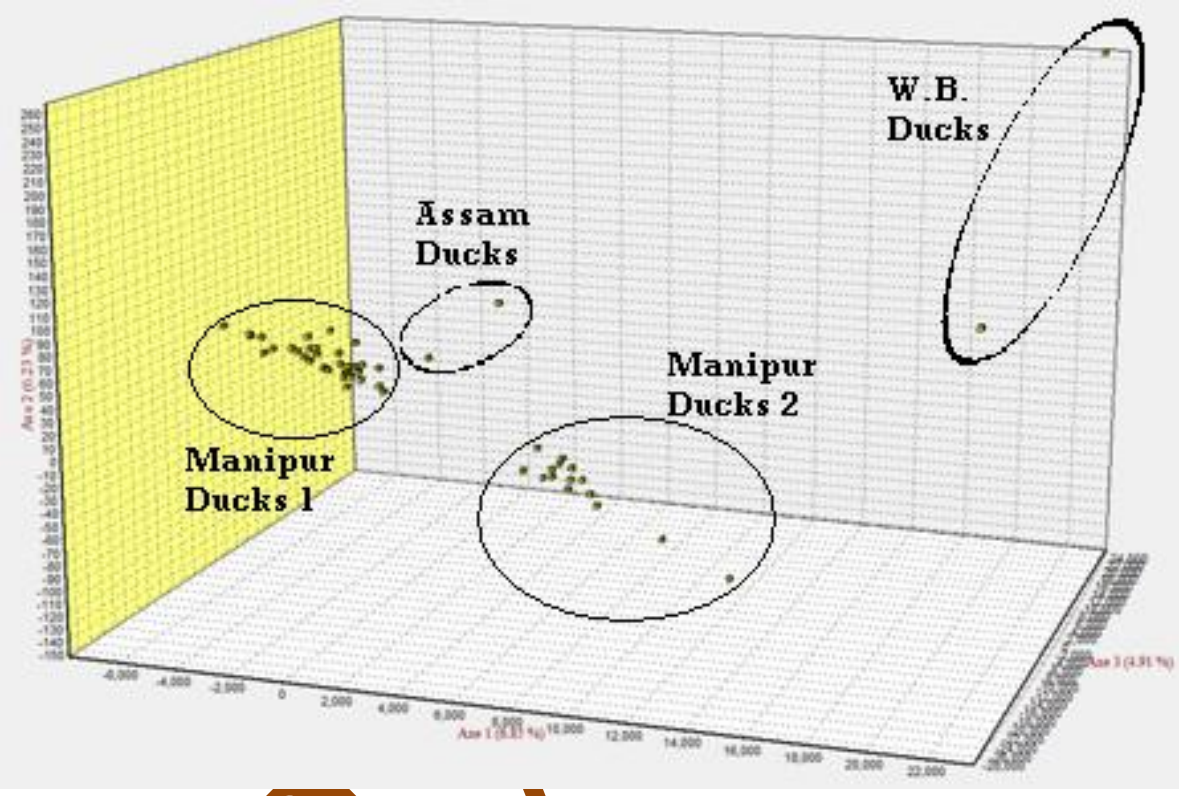


Fig. 3. Correspondence analysis for clustering of Muscovy duck.

Xiao *et al.* (1999) carried out studies on five Fujian duck breeds and two reference duck breeds of China. They found a total of 371 alleles over 32 microsatellite loci with a mean number of alleles per locus as 11.72. The values obtained in the present study are also very similar (11.46) obtained from 24 microsatellite loci. The effective numbers of alleles in the present study were 5.141 to 6.961 which are again very similar to the values of the present study meaning thereby that most of the alleles in Muscovy duck are at low frequency. The average heterozygosity obtained in the present study ranged from 0.512 to 0.70 which is similar to the values obtained in the present study.

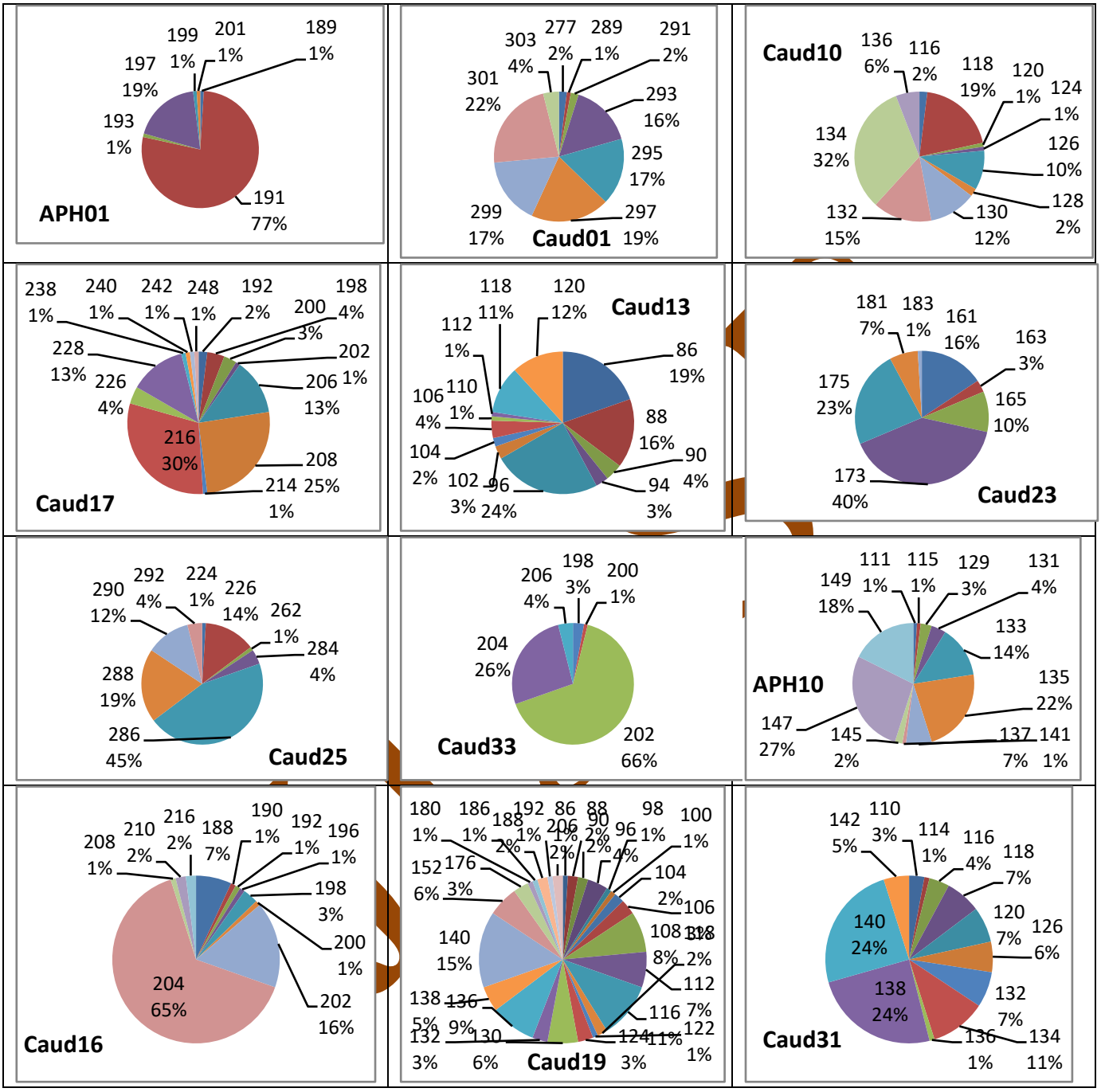
Yan *et al.* (2008) carried out diversity analysis of Muscovy ducks and four other duck breeds of China (*Anas platyrhyncos*). They reported that the heterozygosity values to be more in the Muscovy ducks compared to the other ducks of China. They reported a heterozygosity value of 0.80 which is significantly higher than those obtained in the present study of Muscovy ducks of India. The reason for obtaining less heterozygosity in Indian Muscovy ducks is that we collected blood samples primarily from Manipur state of India where the number of Muscovy ducks is significantly higher and the geographical area over which the samples were collected was not large. The numbers of Muscovy ducks in China are very large and fairly distributed over large geographical area while in

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

India the Muscovy ducks are not separately reared and are few in number in the commercial duck (*Anas platyrhyncos*) rearing states of India.

The data analysis suggests that sufficient gene and genotypic diversity exists at all the 24 heterologus microsatellite loci utilized in the present study. The study reveals that the selected loci could be used for diversity analysis in Muscovy ducks. The effective population size of the Muscovy ducks is fairly large. Separate breeding programs and conservation strategies may be needed for duck diversity conservation in India as Muscovy ducks are not preferred because of the slow growth rate. However, the Muscovy meat is preferred over the table duck meat as it is lean and has less fat content.

(IJIASE) 2016, Vol. No. 2, Jan-Dec e-ISSN: 2454-9258, p-ISSN: 2454-809X



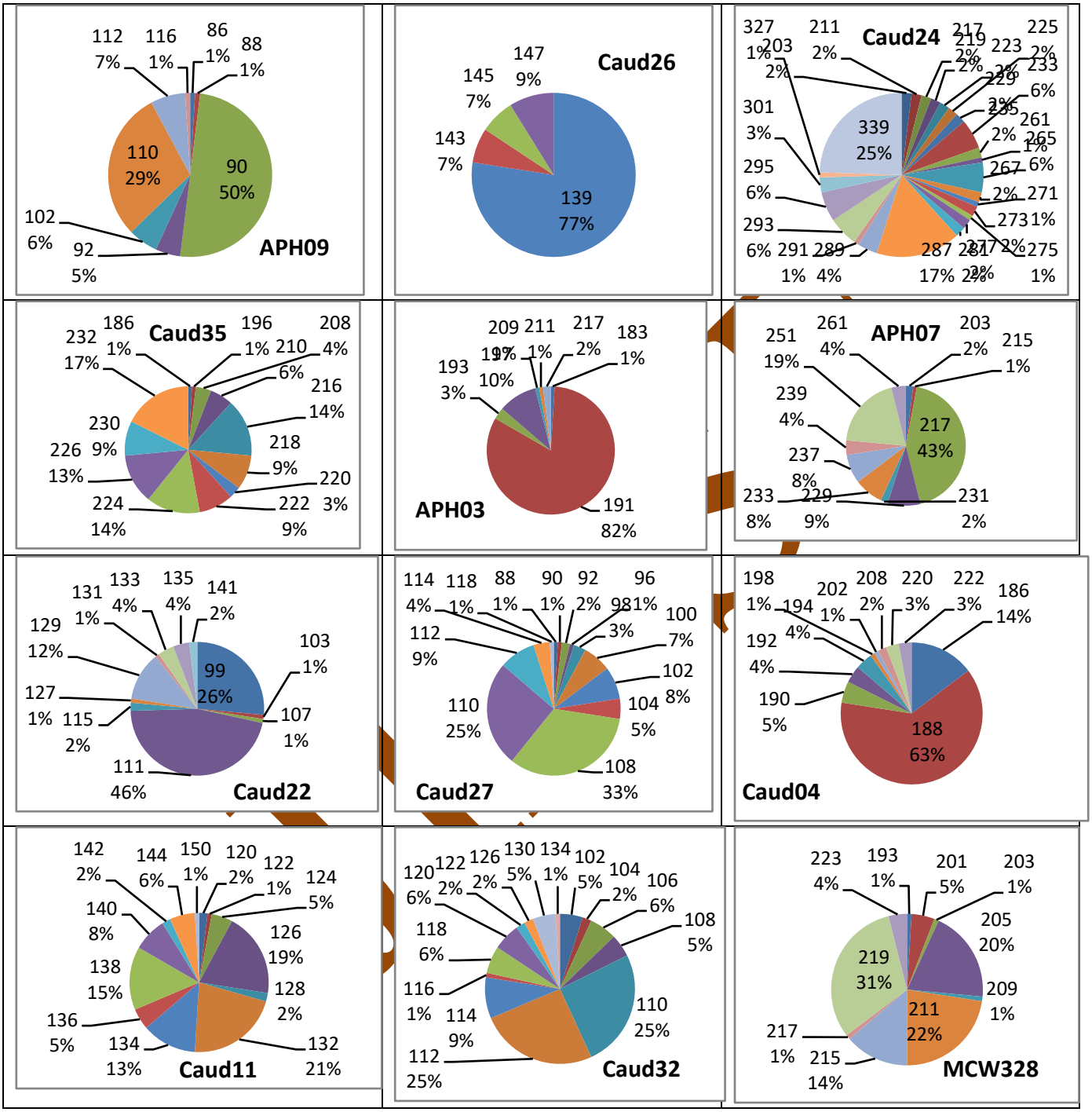


Fig. 4. Allelic distribution at different microsatellite loci.

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