Population structuring of land and coastal ducks (*Anas platyrhynchos*) using microsatellite markers

Uma Gaur[1,5], Madhu Sudan Tantia[2], Bina Mishra[3], S. T. Bharani Kumar[4], Ramesh Kumar Vijh[2] and Ashok Chaudhury[1,*]

[1]Department of Bio & Nano Technology, Bio & Nano Technology Centre, Guru Jambheshwar University of Science and Technology, Hisar 125 001, India
[2]National Bureau of Animal Genetic Resources, Karnal 132 001, India
[3]Indian Veterinary Research Institute, Bareilly 243 122, India
[4]International Atomic Energy Agency, Seibersdorf Laboratories Vienna, Austria
[5]Present address: Sichuan Agricultural University, Wenjiang, Chengdu 611130, Sichuan Province, People's Republic of China

Twenty-four polymorphic and ubiquitously distributed microsatellite loci were utilized to genotype four populations of indigenous Mallard ducks in India using Khaki campbell as an out-group. The data was used to establish population parameters and genetic relationship among the populations. All the selected loci exhibited high polymorphic information content and gene diversity. *F*-statistics revealed population structure in four indigenous duck populations. There was not much differentiation among the duck populations along coastline, while land birds were found to be distinct from coastline ducks. The data presented here will be useful for undertaking duck improvement programmes in future.

Keywords: Duck, genetic diversity, microsatellite, polymorphism.

AVIAN species play a vital role in livelihood of human beings throughout the world, especially in developing countries. Backyard poultry provides livelihood to large and small farmers while the poultry industry has a lead role in raising the economic resources of the country, especially the agricultural sector. The domestic poultry fowls are typically members of the order *Galliformes* (such as chicken and turkeys) and *Anseriformes* (waterfowls such as ducks and geese). Recent publication of world watch list of domestic animal biodiversity (WWLDAD, www.fao.org) reveals 14 avian species (87.5%) and 1049 avian breeds (16.4%) distributed all over the globe. The total population of ducks in India is 10 million and it ranks second in the world after Indonesia.

Taxonomically ducks belongs to the order *Anseriformes*, and family Anatidae that diverged from the chicken (*Galliformes*) 110 million years ago. The mallard (*Anas platyrhynchos*) is the most recognizable of all ducks. It is a dabbling duck, which breeds throughout the coastal regions of India. The Northeast regions of India also have a substantial number of these mallards. India inhabits both mallards as well as muscovy ducks distributed along the coastline, and also in the land-locked areas. These two are believed to be the ancestor of all domestic ducks. The mallards are variable in colour, size and gait. There have been no well-defined breeds of India and the present study was designed to understand the diversity and relationship among different populations of ducks.

The characterization and conservation of ducks assume prime importance as they provide food security to the rural folk. These locally adapted ducks are useful biodiversity resources as they are important genetic reservoirs essential for facing the future challenges of disease resistance and better quality meat. Microsatellite has been the marker of choice for diversity and relationship analysis among different species of poultry and livestock including buffalo, camel and horse[1–6]. Although many reports have used microsatellite for studying the genetic diversity of chickens of Indian origin[1,4,5], jungle fowl[7], Chinese and Japanese origin[8–14] and Korean chickens[15], no reports are available on Indian ducks.

The genetic diversity analysis to establish population parameters and genetic relationship among 15 chicken breeds of India was carried out, utilizing 25 highly polymorphic ubiquitously distributed microsatellite loci[1]. Also, 25 microsatellite markers were utilized to genotype indigenous poultry breeds in two different studies[4,5]. The genetic evidences from red jungle fowl collected on the basis of both microsatellite and mitochondrial DNA indicated the clear separation of *Gallus gallus domesticus* from *Gallus gallus murghi* and *Gallus sonneratii*[16].

There has been no systematic study of mallards in India, although a few reports relating to the ducks of Chinese origin[17–24] are available. In the present study, random samples were collected from four duck populations of West Bengal, Odisha, Tamil Nadu and Jharkhand. Khaki campbell (commercial duck) was taken as an out-group.

*For correspondence. (e-mail: ashokchaudhury@hotmail.com)

Materials and methods

Sample collection and molecular techniques

Sample collection (Figure S1, see Supplementary Information online), DNA extraction[25], quality and quantity check, primer selection, PCR amplification and genotyping for 24 microsatellite markers were carried out as reported earlier[26].

Statistical analysis

The data obtained on these 24 microsatellite loci was analysed to obtain the expected and observed heterozygosity values in the 5 populations using POPGENE software[27]. The software FSTAT 2.9.1 was used[28] for computing values of standard genetic diversity indices, their variances and pairwise estimates of F_{ST}. The F statistics values F_{IS}, F_{IT} and F_{ST} were estimated using Jackknifing over loci and the confidence interval generated using 10,000 permutations with the GDA software[29]. The number of migrants (N_m) was estimated using

$$N_m = 0.25(1 - F_{ST})/F_{ST}.$$

The Hardy Weinberg equilibrium was tested using X^2 and G^2 statistics.

$$X^2 = \sum (O - E)^2 / E,$$

where O is the observed heterozygosity and E is expected heterozygosity. The log likelihood ratio was calculated as

$$G^2 = 2 \times O \times \ln(O/E).$$

The Bayesian analysis for clustering and inferring populations of Indian ducks was carried out using software structure[30].

Nei's standard genetic distances were estimated using POPGENE. The molecular variance (AMOVA) was analysed using the software ARLEQUIN (version 3.0). The correspondence analysis was carried out using genetix (ver. 4.05) software (www.genetix.univ-montp2.fr/genetix/genetixhtml).

Results

The four indigenous duck populations along with Khaki campbell (outgroup) were found to be highly variable at all the microsatellite loci. The number of alleles varied from 4 to 38 in various loci. The minimum number of alleles (4) was observed for CAUD33 and the maximum number (38) for CAUD24. The mean number of alleles per locus was 11.29 (Table 1). However most of the alleles were in low frequency which is reflected by a dramatic decrease in the effective number of alleles. The mean effective number of alleles (4.26) represents the number of alleles that cannot be lost due to chance.

The polymorphic information content (PIC) values for the 24 loci studied were quite high and ranged from 0.26 (MCW328) to 0.89 (CAUD24) (Table 1). The PIC values closely resembled the values expected for heterozygosity and this can be attributed to the large number of alleles at very low frequencies. The observed heterozygosity values for various loci ranged between 0.05 (MCW328) and 0.73 (CAUD19) with a mean value of 0.40 ± 0.03. The high values for these loci make them fit for diversity analysis. The mean heterozygosity in the present study was 0.40 ± 0.03. The mean PIC of all the loci was 0.56 ± 0.03. The values for each of the five populations are given in Table 2. The Khaki campbell taken in the present study as an out-group exhibited observed heterozygosity of 0.40 ± 0.03 and its F_{IS} is 0.24, which is quite high. The number of alleles, the effective number of alleles, the observed heterozygosity and the expected heterozygosity for each locus, in each population are given in Table 1.

The observed population-wise heterozygosity, expected heterozygosity and gene diversity are presented in Table 2. The West Bengal duck population had the highest heterozygosity value of 0.48 ± 0.03 followed closely by Khaki campbell. Heterozygosity was least in the Tamil Nadu duck. These values were significantly less than the expected heterozygosity in all the populations pointing towards the deficiency of heterozygotes. This could be attributed to non-random union of gametes and existence of population structure. The estimates of F statistics, i.e. F_{IS}, F_{IT} and F_{ST} (representing inbreeding at the loci, total inbreeding and population differentiation respectively), were positive except for one locus CAUD22, which showed a negative value for F_{IS}. The calculated F_{ST} estimates provide a measure of population differentiation among the duck populations. The values were highest for CAUD22 (0.53) and MCW328 (0.52) indicating that these loci have the highest differentiation power among the microsatellite loci studied (Table 1).

The population-wise F_{ST} values and the effective number of migrants exchanged between them are given in Table 3. The minimum population differentiation, i.e. maximum germplasm movement was between Odisha and Tamil Nadu populations as there is a large value of effective number of migrants (6.13) between these two populations. The F_{IS} values for all the five populations were significantly different from zero. It also supported the existence of population structure in all four duck populations. The F_{IS} values were smallest in West Bengal ducks (0.22). None of the duck populations studied had a negative F_{IS} value. The gene diversity for all the populations was also very high and ranged from 0.53 ± 0.03 (Khaki campbell) to 0.61 ± 0.09 (West Bengal ducks).

Table 1. Number of alleles, *F* statistics, number of migrants, observed and expected heterozygosity and polymorphic information content (PIC) in Indian duck populations using 24 microsatellite markers

Locus	No. of alleles	Effective no. of alleles	F_{IS}	F_{IT}	F_{ST}	N_m	Ho	He	PIC
APH01	5	1.88	0.06	0.43	0.39	0.39	0.27	0.47	0.28
Caud01	19	3.45	0.11	0.14	0.04	6.62	0.61	0.71	0.68
Caud10	5	1.53	0.12	0.18	0.06	3.73	0.29	0.35	0.33
Caud17	15	4.86	0.73	0.76	0.14	1.56	0.19	0.80	0.68
Caud13	11	3.70	0.07	0.24	0.18	1.13	0.56	0.73	0.60
Caud23	8	4.22	0.20	0.30	0.13	1.72	0.53	0.76	0.67
Caud25	8	2.41	0.52	0.54	0.06	4.24	0.27	0.59	0.55
Caud33	4	2.10	0.11	0.18	0.08	2.87	0.43	0.53	0.48
APH10	8	2.20	0.21	0.39	0.23	0.83	0.33	0.55	0.42
Caud16	10	3.08	0.37	0.48	0.17	1.25	0.35	0.68	0.56
Caud19	29	13.68	0.11	0.21	0.11	2.00	0.73	0.93	0.82
Caud31	15	3.01	0.12	0.18	0.07	3.20	0.55	0.67	0.62
APH09	9	5.72	0.27	0.37	0.14	1.54	0.52	0.83	0.71
Caud26	7	3.16	0.62	0.67	0.11	2.08	0.23	0.69	0.61
Caud24	38	17.07	0.31	0.35	0.05	4.67	0.62	0.94	0.89
Caud35	11	4.14	0.37	0.51	0.22	0.87	0.37	0.76	0.59
APH03	6	2.27	0.64	0.67	0.09	2.41	0.18	0.56	0.51
APH07	12	3.02	0.58	0.63	0.10	2.17	0.25	0.67	0.60
Caud22	9	2.70	-0.09	0.48	0.53	0.23	0.33	0.63	0.30
Caud27	9	3.25	0.11	0.23	0.13	1.62	0.53	0.69	0.60
Caud04	13	5.12	0.06	0.16	0.11	2.14	0.68	0.81	0.72
Caud11	7	3.50	0.17	0.40	0.28	0.66	0.43	0.72	0.52
Caud32	7	4.00	0.17	0.46	0.35	0.47	0.41	0.75	0.49
MCW328	6	2.13	0.79	0.90	0.52	0.24	0.05	0.53	0.26
Mean	11.29 ± 1.53	4.26 ± 0.73	0.28	0.41	0.18	2.03	0.40 ± 0.03	0.68 ± 0.03	0.56 ± 0.03

Table 2. Population-wise gene diversity in Indian ducks

Duck population	Ho	He	F_{IS}	Gene diversity
West Bengal	0.48 ± 0.03	0.60 ± 0.02	0.22	0.61 ± 0.09
Odisha	0.39 ± 0.03	0.58 ± 0.03	0.35	0.59 ± 0.03
Khaki campbell	0.40 ± 0.03	0.53 ± 0.03	0.24	0.53 ± 0.03
Jharkhand	0.39 ± 0.03	0.57 ± 0.03	0.32	0.57 ± 0.03
Tamil Nadu	0.37 ± 0.03	0.54 ± 0.03	0.32	0.55 ± 0.03

Ho, observed heterozygosity; He, expected heterozygosity.

Table 3. Population-wise F_{ST} values and number of migrants in Indian ducks

Duck population	West Bengal	Odisha	Khaki campbell	Jharkhand	Tamil Nadu
West Bengal	–	2.02	0.62	0.93	1.18
Odisha	0.11	–	0.81	1.17	6.13
Khaki campbell	0.29	0.24	–	0.99	0.73
Jharkhand	0.21	0.18	0.20	–	0.92
Tamil Nadu	0.17	0.04	0.26	0.21	–

Number of migrants (above diagonal); F_{ST} values (below diagonal).

The mean number of effective migrants (2.03) per generation was calculated on the basis of F_{ST} values and was quite high as the N_m values are less than 1 in distinct populations (Table 1) indicating quite high gene flow among the duck populations. The increased gene flow is because of the continuity of populations and introduction of germplasm for improvement in egg production. The Hardy-Weinberg equilibrium of five populations for all the 24 loci was tested using χ^2 test and the results have been explained (Table S2, see Supplementary Information online).

In West Bengal duck populations, 21 out of 24 loci deviated from Hardy-Weinberg equilibrium using χ^2 test while 3 loci (Caud23, Caud33 and Caud11) showed no significant deviation. In Odisha duck population, 20 out of 24 loci deviated from Hardy-Weinberg equilibrium while

Table 4. Population-wise genetic distances and identities in five duck populations

Duck population	West Bengal	Odisha	Khaki campbell	Jharkhand	Tamil Nadu
West Bengal	–	0.80	0.46	0.60	0.70
Odisha	0.22	–	0.60	0.69	0.93
Khaki campbell	0.78	0.51	–	0.68	0.59
Jharkhand	0.51	0.37	0.39	–	0.64
Tamil Nadu	0.36	0.07	0.53	0.44	–

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for five duck populations.

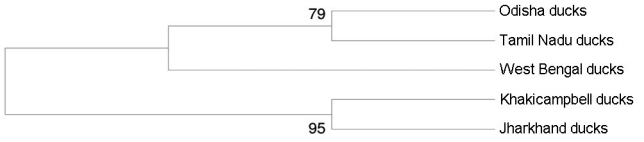

Figure 1. Dendrogram of five duck populations. The values at node depict bootstrap values are based on 1000 replicates.

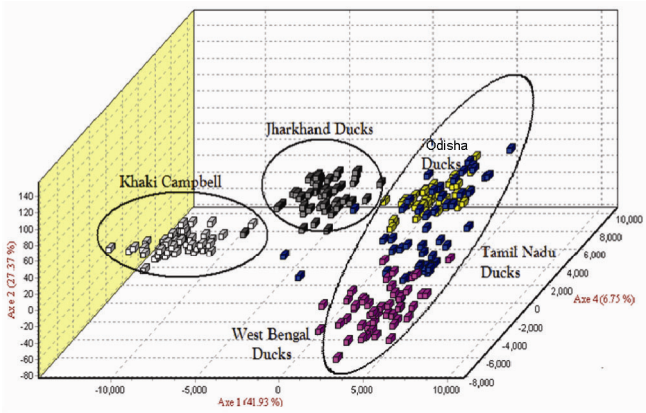

Figure 2. Correspondence analysis for clustering individuals of duck populations.

4 loci (Caud10, Caud23, Caud22 and Caud11) displayed no significant deviation. In Khakhi campbell duck population, 14 out of 24 loci studied deviated from Hardy-Weinberg equilibrium while 10 loci (APH01, Caud01, Caud10, Caud13, Caud33, Caud19, Caud22, Caud04, Caud32 and MCW328) had no significant deviation. In Jharkhand duck population, 16 out of 24 loci deviated from Hardy-Weinberg equilibrium while no significant deviation was observed in 8 loci (Caud10, Caud13, Caud33, APH10, Caud31, Caud22, Caud04 and MCW328). In Tamil Nadu duck population, 16 out of 24 loci deviated from Hardy-Weinberg equilibrium while 8 loci (APH01, Caud10, Caud13, Caud33, APH10, Caud22, Caud11 and Caud32) showed no significant deviation.

The Nei's standard genetic distance D_s was estimated among the populations (Table 4). The upper matrix represents the identity among the populations while the lower matrix represents the genetic distance estimates among the populations. The neighbour joining algorithm was used to construct dendrogram. The strength of the nodes was tested by bootstrap over loci (Figure 1). The dendrogram revealed Odisha and Tamil Nadu ducks to be closely related with bootstrap value of 79, West Bengal ducks joined them later. The Jharkhand duck and Khaki campbell join together at another node with a bootstrap value of 95. This can be attributed to breeding and usage of Khaki campbell birds in Jharkhand area for improvement in egg production.

The AMOVA revealed that a total of 19.76% variation was attributed to variance among population while the rest was between individuals within populations. Thus, individuals within the populations contributed more to variability than between populations. The correspondence analysis revealed three distinct clusters (Figure 2). The out-group Khaki campbell was distinct and all the individuals clustered together. The Jharkhand ducks formed another cluster, while the three populations along the east coastline of India clustered together. Thus, on the basis of geographical proximity or distance, there are three distinct clusters in the Indian duck populations i.e. land birds (Jharkhand ducks), out-group birds (Khaki campbell ducks), and coastal birds (West bengal, Tamil Nadu and Odisha ducks).

The Bayesian analysis for clustering and inferring populations of Indian ducks was carried out using software program STRUCTURE. The duck populations of India are not contained in a particular geographical location, but are present along the east coastline and hence defining the population is a subjective issue. Model-based structure analysis utilizing genetic data obtained on coastline as well as inland birds was carried out. The graphical clustering of populations is shown in Figure 3. The STRUCTURE software implements a model-based approach. For the continuity of duck populations (there being no strict physical barriers or isolation of the populations), the admixture model was employed. In this present data analysis, ten independent runs of Gibbs sampler for each value of K (K = 2, 3, 4, 5) were run. The results presented are based on a burnin value of 50,000 followed by recording of 50,000 MCMC (Monte Carlo Marcov Chain) simulations. To choose an appropriate value of K for this model, the inference for the number of populations is estimated using the formula probability Pr (K/X). From the estimates of P_r (K/X), shown in the last

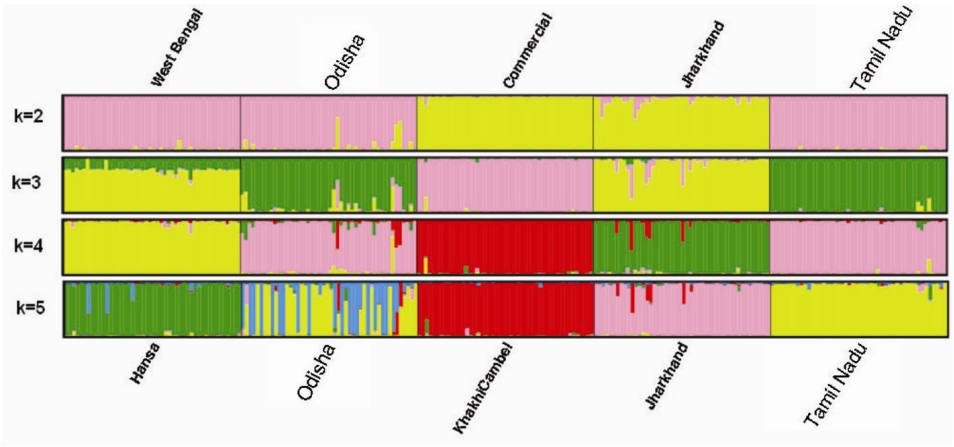

Figure 3. Bayesian analysis for individuals using structure. The admixture model with $K = 5$ is substantially better then models with smaller value of K (2, 3, 4).

Table 5. Inferring the value of K, the number of populations, for the Indian duck population data

K	log $P(X/K)$	$P(K/X)$
2	−15694.8	0.049
3	−14726.8	0.042
4	−13898.2	0.035
5	−13629.8	0.033

column of Table 5, it is clear that the models with $K = 2$, 3 and 4 are completely insufficient to model the data and the model with $K = 5$ is substantially better than models with smaller value of K (2, 3, 4).

Discussion

The present results of microsatellite markers agree with studies carried out in Chinese ducks[22,24,31]. A comparison of the locus-wise results of the Indian and Chinese duck populations revealed that microsatellite markers were highly polymorphic for Indian ducks and the different values are quite comparable between the two sets of populations[20,21]. In comparison to Chinese ducks, the observed heterozygosity values and the polymorphic information content were higher in Indian ducks, owing to the high genetic diversity.

The mean heterozygosity in the present study was 0.40 ± 0.03. The mean PIC of all the loci was 0.56 ± 0.03. This is attributed to mating of individuals who are more closely related to one another than the average relationship among the population members. A similar observation in 10 Chinese indigenous egg-type duck breeds was reported, where the genetic structure and diversity was studied using 29 microsatellite markers[32]. All populations showed high levels of heterozygosity. These results differ from another report where the observed heterozygosity values were lower than the expected heterozygosity values, which pointed towards higher selection pressure and medium differentiation in 26 Chinese indigenous duck breeds across China[33]. In a similar study, the mean polymorphic information content was 0.76 and the mean heterozygosity was 0.79, which indicated a very high polymorphism and genetic diversity in 6 Chinese duck population, for a set of 20 microsatellite markers[34]. The F-statistic analysis carried out in a study on chicken breeds showed the range of F_{ST} from 0.021 to 0.26, which is quite low compared to the present study; however they have utilized an entirely different set of microsatellite markers and that too of Chinese origin[10].

In a similar study of application of microsatellite markers, 5 microsatellite loci have been employed for the paternity index measurement of the parentage-offspring relationships, involving 12 half-sibling families, and the average pedigree error of less than 10^{-3} has been reported among Chinese egg-laying ducks[35]. The genetic diversity, origin, differentiation and relationships in four Chinese indigenous duck breeds, having unique gene pools, were studied using microsatellite markers so as to provide molecular data for pure breeding, cross-breeding and preservation of important genetic resources[23]. A low degree of genetic differentiation was found among the four breeds studied and a significantly high level of variation was observed among individuals within the same breeds. These F_{ST} results suggested a relatively low gene flow between different breeds and, equivalently, a relatively high reproductive isolation within the same ones. Fifty-nine microsatellite markers are used for hybrid classification studies involving endemic Florida mottled duck (*Anas fulvigula fulvigula*) and invasive mallards (*Anas platyrhynchos*)[36]. Markers developed in this study can be used in conjunction with existing markers to robustly classify hybrids and to assess and monitor the genetic dynamics of introgression between these waterfowl species.

In our earlier report of genetic relationship among Indian duck populations[26], the genetic distance studies based on 24 microsatellite loci exhibited ample diversity among the population. All the genetic distances pointed towards large differentiation between the coastal ducks and the land ducks of India. The homogeneity of the coastal ducks, especially of Odisha and Tamil Nadu, reveals free gene flow among the populations and very little to no structuring of the other duck populations. The genetic differentiation between the land and coastal duck populations points to their being separate entities.

Conclusion

The relationship among duck populations in India has been examined using genetic distances in one of our previous reports which is further explained and corroborated in the present study. Clear differentiation between the land and coastal area ducks reflects the need to consider the two as distinctive populations and the breeding plans need to be devised accordingly. The free gene flow among coastal birds reveals homogeneity among the populations. The free gene flow also reflects lack of genetic structure among the coastal ducks and this can be attributed to negligible selection or breeding for specific purposes. The ducks of Jharkhand are distinct from other duck populations and there is a limited gene flow between the ducks inhabiting the land and coastline. There is sufficient diversity in indigenous ducks and this can be exploited for selection and breeding programmes in future.

1. Tantia, M. S., Vijh, R. K., Bharani Kumar, S. T., Mishra, B. and Ahlawat, S. P. S., Genetic diversity analysis of chicken breeds of India. *Indian J. Anim. Sci*., 2006, **76**, 1033–1038.
2. Vijh, R. K., Tantia, M. S., Mishra, B. and Kumar, S. T., Genetic diversity and differentiation of Dromedarian camel of India. *Anim. Biotechnol*., 2007, **18**, 81–90.
3. Vijh, R. K., Tantia, M. S., Mishra, B. and Bharani Kumar, S. T., Genetic relationship and diversity analysis of Indian water buffalo. *J. Anim. Sci*., 2008, **86**, 1495–1502.
4. Ahlawat, S. P. S., Vijh, R. K., Tantia, M. S., Mishra, B. and Bharani, S. T., Genetic diversity analysis of five chicken breeds of India. *Kor. J. Genet*., 2007, **29**, 121–128.
5. Ahlawat, S. P. S., Vijh, R. K., Mishra, B., Bharani, S. T. and Tantia, M. S., Genetic relationship in chicken breeds using molecular co-ancestry information. *Asian Australas. J. Anim. Sci*., 2008, **21**, 6–10.
6. Khanshour, A. M., Juras, R. and Cothran, E. G., Microsatellite analysis of genetic variability in Waler horses from Australia. *Aust. J. Zool*., 2013, **61**, 357–365.
7. Romanov, M. N. and Weigend, S., Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. *Poultry Sci*., 2001, **80**, 1057–1063.
8. Chen, H. J., Yue, Y. S., Fan, X. Z., Zhang, C. S. and Du, L. X., Analysis of genetic diversity of Shandong indigenous chicken breeds using microsatellite markers. *Acta Genet. Sin*., 2003, **30**, 855–860.
9. Chen, G. H. *et al.*, Cluster analysis of 12 Chinese native chicken populations using microsatellite markers. *Asian Australas. J. Anim. Sci.*, 2004, **17**, 1047–1052.
10. Wu, C. H. X. *et al.*, Analysis of genetic diversity among Chinese native chicken breeds using microsatellite markers. *Acta Genet. Sin.*, 2004, **31**, 43–50.
11. Osman, S. A. M., Sekino, M., Nishibori, M., Yamamoto, Y. and Tsudzuki, M., Genetic variability and relationship of native Japanese chicken assessed by microsatellite DNA profiling-focusing on the breeds established in Kochi prefecture, Japan. *Asian Australas. J. Anim. Sci*., 2005, **18**, 755–761.
12. Osman, S. A. M. *et al.*, The genetic variability and relationships of Japanese and foreign chickens assessed by microsatellite DNA profiling. *Asian Australas. J. Anim. Sci*., 2006, **19**, 1369–1378.
13. Liu, G. Q., Jiang, X. P., Wang, J. Y. and Wang, Z. Y., Correlations between heterozygosity at microsatellite loci, mean d_2 and body weight in a Chinese native chicken. *Asian Australas. J. Anim. Sci.*, 2006, **19**, 1671–1677.
14. Chang, G. B. *et al.*, Genetic diversity of wild Quail in China ascertained with microsatellite DNA markers. *Asian Australas. J. Anim. Sci.*, 2007, **20**, 1783–1790.
15. Kong, H. S. *et al.*, Genetic variations and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. *Asian Australas. J. Anim. Sci.*, 2006, **19**, 1546–1550.
16. Kanginakudru, S., Metta, M., Jakati, R. D. and Nagaraju, J., Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. *BMC Evol. Biol*., 2008, **8**, 174.
17. Maak, S., Neumann, K., Lengerken, V. G. and Gattermann, R., First seven microsatellites developed for the Peking duck (*Anas platyrhynchos*). *Anim. Genet.*, 2000, **31**, 233.
18. Stai, S. M. and Hughes, C. R., Characterization of microsatellite loci in wild and domestic muscovy ducks (*Cairina moschata*). *Anim. Genet.*, 2003, **34**, 387–389.
19. Genet, C., Vignal, A. and Larzul, C., Isolation and characterization of microsatellite genetic markers from Peking and Muscovy ducks. *Brit. Poultry Sci.*, 2003, **44**, 794–795.
20. Tang, Q. P., Li, H. F., Tu, Y. J., Chen, K. W., Yang, N., Ma, Y. H. and Zhao, D. W., Analysis of genetic diversity of the domestic concern duck breeds in China. *J. Northwest Sci-Tech. Univ. Agricul. Forestry*, 2007, **35**, 47–52.
21. Li, H. F., Li, B. C., Ma, Y. H., Tang, Q. P., Chen, K. W. and Tu, Y. J., Molecular genetic diversity of Fujian domestic duck breeds. *Ying Yong Sheng Tai Xue Bao*, 2007, **18**, 463–466.
22. Su, Y., Loing, R., Chen, G., Wu, X., Xie, K. and Wan, J., Genetic analysis of six endangered local duck populations in China based on microsatellite markers. *J. Genet. Genomics*, 2007, **34**, 1010–1018.
23. Su, Y. and Chen, G. H., DNA microsatellite analysis of genetic diversity among Chinese indigenous laying-type ducks (*Anas platyrhynchos*). *Czech J. Anim. Sci.*, 2009, **54**, 128–135.
24. Qu, L. J., Liu, W., Yang, F. X., Hou, Z. C., Zheng, J. X., Xu, G. Y. and Yang, N., Origin and domestication history of Peking ducks determined through microsatellite and mitochondrial marker analysis. *Sci. China Series C: Life Sci.*, 2009, **52**, 1030–1035.
25. Sambrook, J., Fritsch, E. F. and Maniatis, T., In *Molecular Cloning: A Laboratory Manual*, Cold Spring Laboratory Press, NY, USA, 1989, 2nd edn.
26. Gaur, U. *et al.*, Genetic relationship among duck populations of India. *Indian J. Anim. Sci.*, 2010, **80**, 444–447.
27. Yeh, F., Boyle, T., Rongcai, Y., Ye, Z. and Xin, J. M., POPGENE: ver. 1.31. A microsoft windows based freeware for population genetic analysis. Quick user's guide. University of Alberta, Edmonton, 1999.
28. Goudet, J., FSTAT version 1.2. A computer program to calculate *F* statistics. *J. Heredity*, 1995, **86**, 485–486.

29. Lewis, P. O. and Zaykin, D., Genetic data analysis: computer program for the analysis of allelic data. Version 1.1. Free program distributed by the authors over the internet from http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php, 2002.
30. Pritchard, J. K., Stephens, M. and Donnelly, P., Inference of population structure using multilocus genotype data. *Genetics*, 2000, **155**, 945–959.
31. Xiao, T. F., Ke, L. Y., Zhang, L. and Jiang, X. B., Genetic diversity of duck breeds: a study with microsatellite markers. *Yingyong Shengtai Xuebao*, 2009, **20**, 190.
32. Hui, F. L., Wei, T. S., Jing, T. S., Kuan, W. C., Wen, Q. Z., Wei, H. and Wen J. X., Genetic diversity and population structure of 10 Chinese indigenous egg-type duck breeds assessed by microsatellite polymorphism. *J. Genet.*, 2010, **89**, 65–72.
33. Liu, W., Hou, Z. C., Qu, L. J., Huang, Y. H., Yao, J. F., Li, N. and Yang, N., Population structure and biodiversity of Chinese indigenous duck breeds revealed by 15 microsatellite markers. *Asian Australas. J. Anim. Sci.*, 2008, **21**, 314–319.
34. Wu, Y., Liu, X. L., Hou, S. S. and Huang, W., Study on genetic diversity of six duck populations with microsatellite DNA. *Asian Australas. J. Anim. Sci*., 2008, **21**, 776–783.
35. Ren, J. *et al.*, Paternity assessment: application on estimation of breeding value in body-weight at first egg trait of egg-laying duck (*Anas platyrhynchos*). *Mol. Biol. Rep*., 2009, **36**, 2175–2181.
36. Seyoum, S. *et al.*, Fifty-nine microsatellite markers for hybrid classification studies involving endemic Florida Mottled Duck (*A. fulvigula fulvigula*) and invasive Mallards (*A. platyrhynchos*). *Conserv. Genet. Resour.*, 2012, **4**, 681–687.

ACKNOWLEDGEMENT. We thank the Department of Biotechnology, New Delhi for financial assistance.

Received 10 December 2014; revised accepted 5 January 2016

doi: 10.18520/cs/v110/i10/1977-1983