Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Poultry Science 5 (3): 107-115, 2011
ISSN 1819-3609 / DOI: 10.3923/ajpsaj.2011.107.115

Genetic Variability of Bali and Alabio Ducks on the Basis of Phenotypic and Microsatellites

Ismoyowati and Dattadewi Purwantini
Faculty of Animal Science, Jenderal Sudirman University, Purwokerto, Indonesia

Corresponding Author: Ismoyowati, Faculty of Animal Science, Jenderal Sudirman University, Purwokerto, Indonesia

ABSTRACT

Identification and characterization of Indonesia local ducks are needed since the information is so important for the Indonesia germ plasm data bank as well as for assisting the genetic improvement program of the species. The identification can be based on qualitative or quantitative phenotype or based on biological molecular through DNA polymorphisms. The research aimed to evaluate the genetic diversity based on the phenotype and genetic relationship using 7 microsatellite primers between Bali and Alabio ducks of Bali and South Borneo region, respectively. A survey has been conducted on farmers of Mengwi (Denpasar, Bali) and Amuntai (Hulu Sungai Utara, South Borneo). Two farmers were chosen randomly on each region and 10 animals were sampled on each farmer making all 40 animals included in the study. The results showed that: the two local ducks differ phenotypically, Bali ducks produced less eggs than did the Alabio and only 5 microsatellite primers were polymorphic. The highest PIC value was of ADL-29 (0.588) with the mean PIC of the Bali and Alabio population of 0.446 and 0.5, respectively. The estimated heterozygosity value on that locus showed a big variation between the two local ducks, i.e. Bali ducks (0.683) and Alabio (0.813). The Hardy-Weinberg Equilibrium test showed that the Bali duck population was a result of random mating while the Alabio was not. The genetic distance evaluation showed that the Bali and Alabio ducks had a genetic relationship of 0.06. The study concluded that the genetic variability of the two local ducks based on phenotype and microsatellite marker was quite high. Bali ducks and Alabio ducks were found to be distantly genetically related.

Key words: Alabio ducks, Bali ducks, heterozygosity, polymorphic information content, genetic distant

INTRODUCTION

There are several kinds of local ducks in Indonesia that are named according to its site origin and have their specific morphological characteristics such as Tegal ducks on the northern coastal areas of Java, Magelang ducks of Magelang region and Mojosari ducks of East Java. The three kinds of ducks live on Java island (Ismoyowati and Purwantini, 2010). Other than the three kinds of ducks mentioned above, there are also Bali ducks on Bali and itik Alabio on South Kalimantan. Some kinds of ducks in Indonesia are the offsprings of cross-breedings between local and imported ducks, therefore, there are several kinds of colors and names for the ducks (Hetzel, 1985; Wilson *et al*., 1997). On the basis of microsatellite marker, there are high genetic variability in the duck population of Java island, Indonesia, with an average PIC value of 0.710±0.099 and heterozygosity value of 0.644±0.096 (Ismoyowati and Purwantini, 2010).

DNA analysis using microsatellite marker is frequently applied for genotypic identification, genetic variability and genetic distance estimations (Chen *et al*., 2003; Romanov and Weigend, 2001). DNA marker has a great possibility to be used to overcome and evaluate the phylogenetic relationship or family relationship between populations that have a very close family relation (Nei and Takezaki, 1996). In a wide sense, microsatellite marker can be applied to estimate great distributions of genetic variability, random genom distribution, to know high level polymorphism and can be used to show co-dominant characteristic inheritances (Groenen *et al*., 2000; Crooijmans *et al*., 1993). The genetic variability information is very important for the selection programme and genetic mapping of a dominant, valuable trait (Siriphovat and Tragoonrung, 1997).

The knowledge of genetic variability of local ducks in Indonesia is extremely important for the conservation consideration of the local ducks. The information on the genetic variability will give great opportunity in the selection in the genetic improvement program which in turn will increase the economical value of the ducks. Huang *et al*. (2008) reported that genetic mapping of duck using microsatellite markers and Single Nucleotide (SNP) was important to be done to know genes that had close relation with the characteristics which had high economy values.

The purposes of this study were: (1) to obtain phenotypic data of Bali and Alabio ducks at the level of small-holder farmers, qualitatively as well as quantitatively, (2) to obtain molecular genetic variability data on the basis of the used microsatellite primary marker and (3) to know the genetic distance between Bali and Alabio ducks.

MATERIALS AND METHODS

The size and sample collection: The materials used were Bali ducks that were raised by the farmers around the territory of Mengwi, Denpasar, Bali and Alabio ducks that were grown around the region of Amuntai, Hulu Sungai Utara, South Kalimantan. Sample collection and laboratory analysis was done from 10th to October 30th 2010. Forty heads of Bali and Alabio ducks from 4 farmers for each kind of duck were taken as samples for blood analysis. The chemicals used were: EDTA as anticoagulant, kit for DNA isolation, 7 kinds of primaries (Table 1), PCR core kit, DNA molecular weight, agarose, TBE solution and ethidium bromide.

Table 1: Primary microsatellites that were used in this study

Primary	Base sequence
ADL-115F	5'-GGA TGA GAA GAA AGG CA-3'
ADL-115R	5'-CAA TGG TGG TTC AGG TAA TC-3'
ADL-209F	5'-GGT TAG CTC CCT CCT TCC AG-3'
ADL-209R	5'-AAG GAA ACA AAG AGA AAT CC-3'
ADL-231F	5'-ACT ATT AGC CTG GGG AGA GC-3'
ADL-231R	5'-TCA CTC CAG CTT GAG ACA GG-3'
APH-23F	5'GGA TGA GAA GAA GAA AGG CA-3'
APH-23R	5'AAG GAA ACA AAG AGA AAT CC-3'
APH-24F	5'GGA TGA GAA GAA GAA AGG CA-3'
APH-24R	5'CAA TGG TGG TTC AGG TAA TC-3'
APH-09F	5'GGA TGT TGC CCC CAC ATA TTT-3'
APH-09R	5'GGA TCA ACC TTA GCT ATC AGT CTC C-3'
APL-2F	5'TTG CCT TGT TTA TGA GCC ATTA-3'
APL-2R	5'TTG CCT TGT TTA TGA GCC ATT A-3'

F: Forward primary; R: Reverse primary. Primary 1-5 (Slavenaite *et al*., 2004), Primary 6 and 7 (Li *et al*., 2006)

Method of study: Survey method at the farmer level and observation in laboratory were used in this study. Phenotypic observations of the duck were conducted toward the colors of feathers, bill, shanks and egg shell. The data of egg production were obtained via interviews with the farmers. Random sampling was applied to select samples for both farmer locations.

DNA isolation: The DNA was isolated using the salting-out method (Miller *et al.*, 1988). The concentration of obtained DNA was measured using spectrophotometer. Because, there was a difference in the concentration, uniformity of concentration at 50 μL prior to PCR was conducted.

PCR and electrophoresis procedure: Seven microsatellite markers (Table 1) were used in this study. The PCR was conducted by using a PCR master mix as much as 15 μL, plus 1 μL of primary forward, 1 μL of primary reserve, 2 μL of DNA and 11 μL of H_2O, therefore, the total volume was 30 μL. The PCR was conducted by using a programmed thermocycler: 94°C for 5 min, 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and 72°C for 5 min. The PCR was conducted in as many as 30 cycles. The results of amplification was separated by using 2% of agarose gel electrophoresis in 1x TBE buffer, using an ethidium bromide coloring. The obtained results were observed using ultra violet radiation and photographed for documentation.

Data analysis: The total and average values of genetic variability of the local ducks were measured based on DNA polymorphism and the average value of Heterozygosity (H). The gen frequency was calculated according to the formula of Pirchner (1981):

$$F_{An} = \frac{\sum locus_{An}}{\sum locus_{A1} + \sum locus_{A2} + \sum locus_{An}}$$

Where:
F_{an} = Frequency of gen A at the-n locus sequence

The calculation of genetic variability was conducted using heterozygosity formula of Nei (1987):

$$h = 1 - \sum_{i=1} x_i^2$$

Where:
h = Heterozygosity
x_i = The ith gen frequency

The average value of Heterozygosity (H) was the average value of h toward the total number of loci or:

$$H = 1 - \frac{\sum_{i=1} x_i^2}{R} h/r$$

Where:
r = The No. of loci being observed

The value of genetic Distance (D) was obtained via the data of allele frequency of all observed loci, using a formula of Nei (1987):

$$D = - \log_e I$$

Where:
I = The value of genetic similarity between two populations, calculated by using a formula:

$$I = \frac{\Sigma X_{ij} Y_{ij}}{\sqrt{X_{ij}^2 Y_{ij}^2}}$$

Where:
X_{ij} = The ith allele frequency ke-I, jth locus, of X population
Y_{ij} = The ith allele frequency at the jth locus of Y population

Polymorphic information content was calculated by the formula:

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}$$

Where:
PIC = Polymorphic information content
Pij = Allele frequency of i marker

RESULTS AND DISCUSSION

The phenotype of bali and alabio ducks: The results of phenotype characteristics of Bali and Alabio ducks in Bali and South Kalimantan were compiled in Table 2. Bali ducks had a various feather colors and egg production relative to those of Alabio. The Alabio ducks had body weight and egg production higher than Bali ducks. The core area of Alabio duck farms was in the village of Alabio, South Amuntai Sub-District, Hulu Sungai Utara District, Kalimantan. Prasetyo and Susanti (2006) reported that Alabio ducks laid their first eggs at the average age of 177±26.2 days at the average body weight of 1693.8±152.1 g head^{-1}, with a yearly (12 months) egg production as many as 248.8±0.7 eggs head^{-1}. The Bali ducks are distributed on Bali island especially in Tabanan, Denpasar and Ubud. Besides being raised for egg production, the white-color Bali ducks are also good meat producers, especially for religious ceremonies and rituals. Egg production was still various, because the farming system was still traditional (the farmers accompanied the ducks where ever they go for feeding) and some of the ducks had already been raised in close houses. The Bali ducks laid their first eggs at the average age of 157 days (Hardjosworo *et al*., 2001).

The results of this study revealed that there was a genetic variability between the two breeds of local ducks, Bali and Alabio. The genetic factor extremely determines production potential and is supported by the proper environmental factors. The results of this study could be used as a data base for the improvements of genetic quality and for the developments of local ducks in Indonesia through selection and mating system. Cherry and Morris (2008) stated that domesticated ducks

Table 2: The data of body size and weight, the colors of feathers, shanks, skin and bill of Bali and Alabio ducks

Observed variable	Bali ducks	Alabio ducks
Body weight (g)	1480±120	1670±150
Yearly egg production	200-260	250-300
The colors of:		
Feathers	(1) totally white without any variation for both sexes. The male ducks had over-grown feathers at the top of head. (2) Variations of chocolate, black and white.	Dominated by greyish, chocolate color blended with yellow spots for females and black spots around their backs for male ducks. The tips of their wings were greenish-blue for females, whereas for males, the color of wing tips was purple-blue. For males, the color of their tail was black, some of their feathers flipped up-side. Black color was found at the top of head.
Bill	(1) Yellow for the white-color ducks, (2) Yellow or black for the ducks whose feather colors were chocolate, black and chocolate-black or black-white combination.	Yellow
Shank	(1). Yellow for the white-color ducks. (2) Yellow or black for the ducks whose feather colors were chocolate, black and chocolate-black or black-white combination.	Yellow
Skin	(1) Rather yellowish and bright, for the white-colored ducks. (2) The color of skin was rather dark-white for the ducks whose feather colors were chocolate, black and chocolate-black or black-white chocolate combination.	Rather yellowish and bright
Body size	Bottle-like, slim, long neck. breast, round abdomen	The body was relatively big, nearly vertical

including Indonesian ducks represented the off springs of mallard (Anas platyrhinchos) and the famous duck in Indonesia as a good egg producer was Alabio (245 eggs year^{-1}). The Bali and Alabio ducks have similar egg production capacity compared to those of Khaki Campbell (240 eggs year^{-1}) and Tsaiya ducks (250 eggs year^{-1}).

DNA Polymorphism of Bali and Alabio ducks based on microsatellite marker: The results of DNA isolation and identification by using 7 kinds of primary microsatellite, yielding five primaries that showed the occurrence of DNA polymorphism of the ducks, whereas two primaries were not identified (Table 3). The ranges of allele size were various, beginning from 100-500 bp, with APH-23 as the largest allele size (100-600 bp) and the smallest was that of ADL-23 (100-300 bp). The number of identified allele from each locus was 2 and 3, the total number of identified allele couple yang from 5 primaries were 12 couples in the whole population, whereas, 2 unidentified primaries were ADL-115 and APH-24. The highest PIC values on the basis of the obtained locus from whole population were the ADL-209 (0.588). Ahmadi *et al.* (2007) reported that from 13 microsatellite markers that were used to amplify the DNA of Pekin ducks (*Anas platyrhynchos*) and Manila ducks (*Cairina moschata*), 4 microsatellites were not identified, 3 microsatellites had a monomorphic trait and 6 microsatellites showed the existence of DNA polymorphism.

The estimation of expected heterozygosity (He) and Polymorphic Information Content (PIC) values was obtained by using the data of each locus of duck population. The values of He were high

Table 3: Seven microsatellite markers that were used in the identification of DNA polymorphism of local ducks

Locus	Allele size (bp)	No. of allele	He	PIC
APL-2	200-500	3	0.850	0.511
APH-09	100-350	2	0.745	0.489
ADL-23	100-300	2	0.744	0.489
ADL-115	Unidentified	0	0.000	0.000
ADL-209	100-400	3	0.863	0.588
APH-23	100-600	2	0.833	0.499
APH-24	Unidentified	0	0.000	0.000
Average*			0.807±0.058	0.515±0.042

He: Expected heterozygosity, PIC: Polymorphic information content, *Average was computed excluding unidentified alleles

Table 4: Genetic variability of Bali and Alabio ducks

Lokus	Parameter	Bali	Alabio
APL-2	N	20.00	21.00
	PIC	0.32	0.576
	He	0.44	0.859
APH-09	N	20.00	21.00
	PIC	0.398	0.489
	He	0.699	0.745
ADL-23	N	13.00	21.00
	PIC	0.426	0.50
	He	0.713	0.75
ADL-209	N	2.00	14.00
	PIC	0.64	0.638
	He	0.88	0.879
APH-23	N	0.00	14.00
	PIC	0.00	0.499
	He	0.00	0.833
PIC population		0.357±0.232	0.540±0.065
He population		0.546±0.343	0.813±0.062

N: No. of identified sample, PIC: Polymorphic information content, He: Heterozygosity estimation

enough, ranged from 0.744 (ADL-23) up to 0.850 (APL-2) and the average value of He of the whole population was 0.807±0.058 (Table 3). The estimation of He value from different loci and duck populations showed a great variation; 0.546±0.343 and 0.813±0.062 (Table 4) for Bali and Alabio ducks, respectively. These values indicated that there was a higher genetic variability in Bali duck population relative to that of Alabio ducks. The high value of He was due to low in-breeding and selection and great number of detected alleles. The great number of detected alleles showed a high variability especially that of Alabio ducks. Li *et al.* (2006) reported that the genetic variability of local ducks in China ranged from 0.514 up to 0.617 with an average of 0.569, whereas, Vafaei *et al.* (2009) reported that the He values of Pekin and Manila (Moscovy) ducks were relatively low; 0.4329 and 0.4069, respectively. The low value of He indicated the occurrence of in-breeding, selection stress and the low number of detected alleles found in each locus, with the average values of 2.22 and 2.44, respectively. Tadano *et al.* (2007) stated that high heterozygosity indicated the occurrence of matings between individuals that did not have any close family relation in the wide, extended population of poultry.

The average values of PIC were 0.446 and 0.683 for Bali and Alabio ducks, respectively. Ismoyowati and Purwantini (2010) reported that the average values of PIC for Mojosari, Magelang and Tegal duck populations were 0.620, 0.699 and 0.76, respectively, whereas the estimated He value for different loci and populations of ducks showed a great variation; 0.509, 0.695 and 0.728 for Mojosari, Tegal and Magelang ducks, respectively. The PIC values indicate the value of a marker that is used to detect polymorphism in a population. The PIC depends on the number of identified alleles and the distribution of allele frequency. Microsatellite marker used in this study was an intra-species marker, namely a marker that can be used for chicken, ducks and other species of water fowl. For chicken, the microsatellite marker is ADL, whereas for ducks and other water fowls the markers are APL and APH. The microsatellite for DNA conservation in poultry can be used for intra-species and there are 20.42% of constant microsatellite loci in the species of chicken and ducks (Huang *et al*., 2005). The results of this study was also supported by Christel *et al*. (2006) who stated that some DNA microsatellite markers were very specific for a certain species, therefore, the result of this study indicated that the DNA samples of Bali and Alabio ducks were not wholly identified by the used microsatellite in this study.

The results of Hardy-weinberg Equilibrium (HWE) test showed that all the identified loci of Bali duck population was in line with the HWE law which meant that Bali duck population was originated from random matings. There was a distracted path of HWE law in the Alabio duck population that was caused by the occurrence of selection and migration. Selection existed because the farmers conducted the selection of dods (day old ducks), adult males or females (hen). Migration occurred because farmers imported some ducks from the outside farm locations with an expectation that egg production would increase. The occurrences of selection and migration might cause the losses of some alleles and the fault of genotyping (Vanhala *et al*., 1998). The results of this study showed that there were some alleles with the value of null (0), therefore, some individuals who had hemizygote genotype were not found (0). The distraction from HWE is also caused nonrandom mating (Tadano *et al*., 2007). The local ducks in Indonesia that live on Java island (Tegal, Magelang and Mojosari) showed a distraction from the HWE and had a relatively far relation one to the other (0.172-0.325) (Ismoyowati and Purwantini, 2010). The results of the genetic distance on the basis of 5 microsatellite markers showed that there was a relatively far family relationship between Bali and Alabio ducks (average D value of 0.016).

CONCLUSION

The conclusions of this study was the genetic variability based on duck phenotype and genetic variability estimation by using microsatellite marker for Bali and Alabio duck population indicated that there was a high variability. The family relation between both kinds of ducks was far, therefore, the genetic materials of both ducks can be used as a basis for the breeding program in order to increase the genetic value of Indonesian local ducks.

ACKNOWLEDGMENT

Appreciation is forwarded to the Directorate General of Higher Education (Direktorat Jenderal Pendidikan Tinggi/DIKTI) who financially funded this study via and in accordance with the letter of "Surat Perjanjian Pelaksanaan Penelitian Fundamental Nomor: Kept. 1590.19/H.236/DT.01.00/2010 tanggal 7 April 2010". Thank you for Suwarno and Agus Susanto who has translated this article in English.

REFERENCES

Ahmadi, A.K., G. Rahimi, A. Vafaei and H. Sayyazadeh, 2007. Microsatellite analysis of genetic diversity in pekin (*Anas platyrhynchos*) and muscovy (*Cairina moschata*) duck populations. Int. J. Poult. Sci., 6: 378-382.

Chen, X.Y., X.L. Chen, L.J. Lu, Y. Zhao, Z.H. Zuo and Y.Z. Lai, 2003. Research for domestic duck resources and the strain breeding. J. Xiamen Univer., 40: 642-646.

Cherry, P. and T.R. Morris, 2008. Domestic Duck Production: Science and Practice. CAB International, Wallingford, Oxfordshire, UK.

Christel, M.E., F. Katia, G. Carine, C. Gaelle, V. Florence and V. Alain, 2006. Microsatellite DNA markers for duck (*Anas platyrhynchos* and *Cairina moschata*). Proceedings of Symposium COA/INRA Scientific Cooperation in Agriculture, Nov. 7-10, Tainan, (Taiwan, R.O.C.), pp: 165-168.

Crooijmans, R.P., A.J. van Kampen, J.J. van der Poel and M.A. Groenen, 1993. Highly polymorphic microsatellite markers in poultry. Anim. Genet., 24: 441-443.

Groenen, M.A., H.H. Cheng, N. Bumstead, B.F. Benkel and W.E. Briles *et al*., 2000. A consensus linkage map of the chicken genome. Genome Res., 10: 137-147.

Hardjosworo, P.S., P.P. Setioko, L.H. Ketaren, A.P. Prasetyo and S. dan Rukmiasih, 2001. The development technology of Indonesia water fowl husbandry. Proceedings of the Water Fowl Workshop, Aug. 6-7, Bogor Agriculture Institute and Centre, pp: 22-41.

Hetzel, D.J.S., 1985. Duck Breeding Strategies the Indonesia Example. In: Duck Production Science and World Practice, Farrell, D.J. and P. Stapleton (Eds.). University of New England, Armidale NSW., pp: 204-233.

Huang, Y., J. Tu., X. Cheng, B. Tang and X. Hu *et al*., 2005. Characterization of 35 novel microsatellite DNA marker from the ducks (*Anas plathyrhynchos*) genome and cross-amplification in other birds. Genet. Select. Evolut., 37: 455-472.

Huang, Y.H., N. Li, D.W. Burt and F. Wu, 2008. Genomic research and application in the duck (*Anas plathyrhynchos*). Poult. Sci. J., 64: 329-341.

Ismoyowati and D. Purwantini, 2010. An estimation of genetic variation in indonesian local duck using microsatellite marker. Asian J. Poult. Sci., 4: 198-204.

Li, H., N. Yang, K. Chen, G. Chen and Q. Tang *et al*., 2006. Study on molecular genetic diversity of native duck breeds in China. Poult. Sci., 62: 603-611.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleate cells. Nucleic Acids Res., 16: 1215-1215.

Nei, M., 1987. Molecular Evolutionary Genetics. 1st Edn., Columbia University Press, New York, USA.

Nei, M. and N. Takezaki, 1996. The root of phylogenetic tree of human population. Mol. Biology Evolut., 13: 170-177.

Pirchner, F., 1981. Populations Genetics in Animal Breeding. W.H. Freeman and Co., San Fransisco.

Prasetyo, L.H. and T. Susanti, 2006. Female breed establishment through mass selection. Research report Centre for Research in Animal Husbandry, Bogor.

Romanov, M.N. and S. Weigend, 2001. Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poult. Sci., 80: 1057-1063.

Siriphovat, V. and S. Tragoonrung, 1997. Genetic similarity estimated in crossbred broiler chickens with rapid technique. Proceeding of the 35th Kasetsart University Annual Conference, Feb. 3-5, Kasetsart University, Bangkhen, Bangkok, pp: 298-303.

Slavenaite, S., D. Butkauskas, A. Sruoga and E. Mozaliene, 2004. Comparative investigations of mallard ducks (*Anas plathyrhynchos*) genomic DNA using chicken and duck specific microsatellite primers. Veterinarija Zootechnika T, 26: 89-92.

Tadano, R., M. Nishibori, N. Nagasaka and M. Tsudzuki, 2007. Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses. Poult. Sci., 86: 2301-2308.

Vafaei, A., G. Rahimi, H. Sayyazadeh and A.K. Ahmadi, 2009. Genetic structure analysis of pekin and mucovy duck population in North of Iran using microsatellite marker. http://lba.zwans.com/fullpapers/10884.pdf

Vanhala, T., M. Tuiskula-Haavisto, K. Elo, J. Vilkki and A. Maki-Tanila, 1988. Evaluation of genetic distances between 8 chicken lines using microsatellite markers. Poult. Sci., 77: 783-790.

Wilson, B.J., D.M. Martin and H. Nott, 1997. Future genetic improvement in pekin type duck. Proceedings 11th European Symposium on Waterfowl, (ESW'97), Nantes, Fraance, pp: 328-334.