



GENETIC DIVERSITY AND POPULATION STRUCTURE OF MANDAR BESAR (*Porphyrio porphyrio*) INHABITING JAVA WETLANDS

Sena Adi Subrata

Faculty of Forestry, Gadjah Mada University. Jl. Agro No.1. Bulaksumur, Yogyakarta 55281.
Correspondence: adisubrata@ugm.ac.id

ABSTRACT

Despite the occurrence of a massive agricultural landscape change in Java, very few reliable studies evaluate its genetic consequence for impacted wildlife species. This study reported the genetic diversity of the Mandar besar (*Porphyrio porphyrio*) in the modified landscape of Java as a backdrop for such an evaluation. Aim of this study was to estimate genetic diversity and genetic structure of the species. I collected eighty-eight blood samples from five a-priory populations (Demak, Jombor, Kendal, Pagak, and Nawan) and genotyped them at ten selected microsatellite loci to estimate allelic richness (R) and expected heterozygosity (H_e), fixation index (F_{ST}) and genetic clusters (K). DNA was isolated using a commercial kit (Invitrogen) and amplified using touch-down polymerase-chain-reaction technique. Allele length was estimated using an automatic sequencer. Pre-analysis showed that all selected loci have null alleles frequency less than 0.2, successful amplification rate more than 80%, no significant linkage between loci and no deviation from HWE. Further analysis employing GDA and FSTAT showed that Demak population has richest genetic diversity in term of R, meanwhile Jombor was the poorest population. Analysis of population structure employing FSTAT and STRUCTURE showed that the population was likely structured into four subpopulations, as showed by FST (0.085; $P < 0.05$) and $K=4$ (ln probability -1725.2+3.92). This study might reveal that genetic diversity and population structure of Mandar besar in Java were still in common level as compared to other aquatic bird species.

Key words:Swamphen, bird, richness, genetic, wetlands

INTRODUCTION

Biodiversity in Asia is threatened by agricultural development (Sodhi et al, 2010). The development resulted in rice field prominence observed in many Asian countries due to high demand of rice grain in this region (Zhao *et al.*, 2006). Likewise, agricultural development in Indonesia resulted in dominance of wet rice field over Java Island (Whitten et al, 1996), at cost of natural wetlands. It possibly threatens biodiversity, particularly for species which inhabits in natural wetlands (Whitten *et al.*, 1996). Reduction of natural wetlands may lead to population decline and restriction of individual exchange between populations and, in turn, lessen genetic diversity of wetland dependent species. Evaluating this effect is essential for encouraging spatial planning that prevents further loss of biodiversity due to agricultural development in Asia, as proposed by Zhao *et al.* (2006).

I applied population genetic study to Purple Swamphen inhabiting Java wetland. The Purple Swamphen is likely to be affected by wetland disturbance because it has restricted local range and poor flight capability (Taylor, 1998). Aim of this study was to estimate genetic diversity and structure of the species.

MATERIALS AND METHODS

Blood samples of eighty eight individuals bird were collected from five locations: Demak, Kendal, Pagak, Jombor and Nawan during February 2009 – January 2010. After captured,

the bird was tagged and released. Collected blood was preserved in 1 ml ethanol. From the blood, DNA was extracted using PureLink™ Genomic DNA kit (Invitrogen). Extracted DNA then was amplified at 10 selected loci employing touchdown PCR technique. Following amplification, fragment analysis was performed. Prior to analysis, data was checked for evidence of null alleles and gametic disequilibrium. Detail of the genetic technique and result of pre-analysis can be found in Subrata & Storch (2012).

Genetic variability of overall and each population were measured in term of mean of allelic richness (Mousadik & Petit, 1996), observed (H_o) and expected (H_e) heterozygosity. I used FSTAT (Goudet, 2005) to calculate R, meanwhile H_e and H_o were measured employing GDA (Lewis & Zaykin, 2011).

Genetic structure was measured in term of fixation index (F_{ST}) using FSTAT (Goudet, 2005). Significance of the genetic structure was calculated after 10,000 permutations and adjusted for Bonferroni correction ($P < 0.005$). Besides by fixation index, genetic structure was also determined by population clustering using STRUCTURE (Pritchard et al, 2000). It was performed involving spatial location of samples to enhance the capability of the program to detect population structure. I calculated estimated membership coefficient for each individual in a cluster (Q) by performing 10 runs x 1,000 batches x 50,000 Monte-Carlo Markov-Chain (MCMC) iterations for each predefined maximum number of cluster (K). I performed MCMC for K=1 to 6. Considering the large number of MCMC iteration, I used STRUCTURE HARVESTER (Earl and von Holdt, 2012) to plot the mean of estimated ln probability of data and its standard deviation for each K and determine the number of cluster after Pritchard et al (2000). At the appropriate K, calculated Q after MCMC simulation then was averaged using CLUMPP (Jakobsson & Rosenberg, 2007) and visualized using DISTRUCT (Rosenberg, 2004).

RESULTS AND DISCUSSION

Genetic diversity

Using ten selected loci for assessing genetic diversity, I found that overall allelic richness (R) was 5.24, calculated based on 71 individuals. Mean H_e was 0.563, slightly lower than H_o (0.564). Observing R of each population, I found that population of Demak had richest genetic diversity ($R=3.45$), meanwhile population of Jombor was the poorest ($R=2.87$). Additionally, overall and each population were in HWE (Table 1).

Table 1. Allelic diversity of overall and each population. R: mean of allelic richness calculated based on 9 individuals (each population) and 71 individual (overall population), H_o : observed heterozygosity, H_e : expected heterozygosity, and HWE: Hardy-Weinberg Equilibrium, n.s. = non significant.

Population	R	H_o	H_e	HWE
Demak	3.45	0.55	0.56	n.s
Jombor	2.87	0.56	0.52	n.s
Kendal	3.23	0.65	0.52	n.s
Pagak	3.5	0.56	0.54	n.s
Nawan	2.95	0.54	0.50	n.s
<i>Overall</i>	5.24	0.564	0.563	n.s.

Comparing to other waterbird species, population of Purple Swamphen inhabiting Java wetland do not indicate a low level genetic diversity. H_e of our population (0.56) was slight higher than its congeneric and endangered species, Takahe (0.53; Grueber *et al.*, 2011), but was similar with H_e of 17 common species of aquatic birds (0.56; Eo *et al.*, 2011). Although the comparison potentially biased due to difference in employed genetic markers, however it roughly indicated that genetic diversity of the Purple Swamphen is still in a common level. The common level of genetic diversity may suggest that loss of genetic variability of our population can be equalized by gene-gaining factors (Amos & Harwood, 1998). Considering that inbreeding depression potentially applies to this species (Lambert *et al.*, 1994), gene exchange between populations possibly plays important role as counterbalance to inbreeding pressure. This possibility may need further research as some study reported a restricted range and weak movement capability of this bird species.

Genetic structure

This study found that Purple Swamphen populations in Java was significantly substructured ($P < 0.05$) at moderate level ($F_{ST} = 0.085$). The population was likely consisting of four genetic clusters (K), as indicated by the lowest mean of estimated \ln probability of data and standard deviation of Bayesian clustering at this K (Fig. 1). The first genetic cluster (presented as a middle grey bar) dominated Nawan population with estimated coefficient membership (Q) almost 1 (Fig. 2). Meanwhile, other three genetic clusters contributed to genetic composition of Kendal, Jombor and Pagak population at various proportions. In opposite to Nawan, Kendal population consisted of all genetic clusters. Individuals of Demak population originated from second cluster (displayed as black bar) and third cluster (displayed as light grey bar) at similar probability as showed by middle value of Q. The second cluster was also found in Pagak population and the third cluster contributed to Kendal population. Population member of Jombor had high probability originating from fourth cluster (displayed as dark grey) and less probability from third cluster. Individuals inhabiting Pagak were most likely to be originated from second cluster and less likely from fourth cluster.

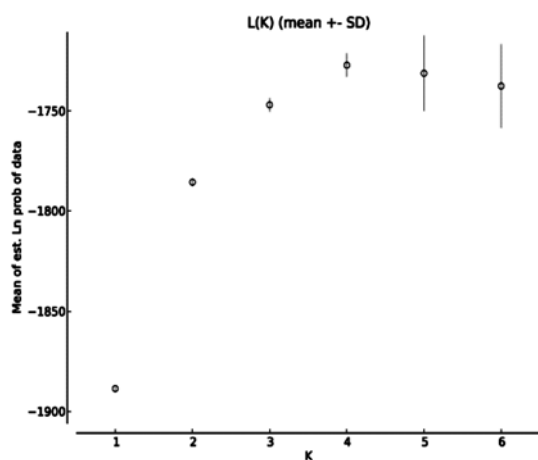


Figure 1. Mean of estimated \ln probability of data for each maximal number of cluster (K). At K=4, mean of estimated \ln probability of data and standard deviation were the lowest.

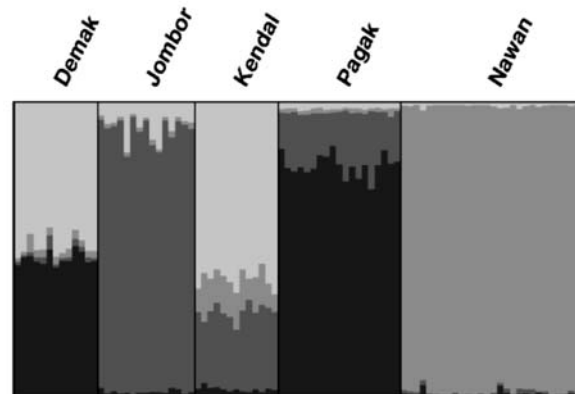


Figure 2. Bar plot of the estimated membership coefficient of each individual (Q) grouped by population. Q ranged from 0 to 1.

Examining population structure, I found supporting evidence to abandon our a priori population structure represented by sampling locations. Although collected from five sampling locations, my samples are most likely originating from four genetically distinct populations (genetic clusters). A cluster dominated Nawan population. It may indicate that this population is less likely sharing their ancestor with other populations. Unlike Nawan, Kendal most probably shared their ancestor with all other populations. Spatially, Nawan is distantly located from other populations; meanwhile Kendal is located in the centre of the population network. It may suggest that spatial distance to other populations influences genetic clustering through successful migration. Observed pattern of population structure indicates the importance of landscape factor in determining individual exchange.

CONCLUSION

Our case shows that recent genetic diversity and population structure of Purple Swamphen are about in the common level. Possibly, gene exchange between populations played important role in determining the level. This possibility raises further questions about bird migration. The question will be interesting because some previous study reported that this species has restricted range and low movement capability.

REFERENCES

- Amos, W., and J. Harwood. 1998. Factors affecting levels of genetic diversity in natural populations. *Phil. Trans. R. Soc. Lond.* (353):177-186
- Earl, D., and B. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4, 359–361.
- Eo, S.H., J.M. Doyle, and J.A. DeWoody. 2011. Genetic diversity in birds is associated with body mass and habitat type. *Journal of Zoology* 283, 220–226.
- Goudet, J. 1995. FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *Journal of Heredity* 86, 485–486.
- Goudet, J. 2005. *FSTAT (ver.2.9.4), a program to estimate and test population genetics parameters*. Available from <http://www2.unil.ch/popgen/softwares/fstat.htm>. Updated from Goudet (1995).
- Grueber, C.E., J.M. Waters, and I.G. Jamieson, I.G., 2011. The imprecision of heterozygosity-fitness correlations hinders the detection of inbreeding and inbreeding depression in a threatened species. *Molecular Ecology* 20, 67–79.
- Jakobsson, M., and N.A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801-1806.
- Lewis, P. O., and D. Zaykin. 2001 *GDA (GENETIC DATA ANALYSIS): Computer Program for the Analysis of Allelic Data (Univ. of Connecticut, Storrs, CT), Version 1.0 d16c*.
- Lambert, D.M., C.D. Millar, S. Anderson, and J.L. Craig. 1994. Single- and multilocus DNA fingerprinting of communally breeding Pukeko: do copulations or dominance ensure reproductive success ?. *PNAS* 91 (20):9641-9645

- Mousadik, A., and R.J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree *Argania spinosa* (L.) Skeels] endemic to Morocco. *TAG Theoretical and Applied Genetics*92, 832–839.
- Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics*155, 945–959.
- Rosenberg, N.A. 2004. Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*4, 137–138.
- Sodhi, N., M. Posa, T. Lee, D. Bickford, L. Koh, and B. Brook. 2010. The state and conservation of Southeast Asian biodiversity. *Biodiversity and Conservation*19, 317–328.
- Subrata, S.A., and I. Storch. 2012. A core set of microsatellite markers identified for use in population genetic studies of Purple Swampphen (*Porphyrio porphyrio*). *Animal Biodiversity and Conservation* (35).1: 23-26.
- Taylor, P.B., 1998. *Rails. A guide to the rails, crakes, gallinules and coots of the world*. Pica Press [u.a.], Robertsbridge, Sussex.
- Whitten, A.J., R.E. Soeriaatmadja, and S.A. Afiff. 1997. *The ecology of Java and Bali*. Oxford University Press, Oxford.
- Zhao, S., C. Peng, H. Jiang, D. Tian, X. Lei, and X. Zhou. 2006. Land use change in Asia and the ecological consequences. *Ecological Research*21, 890–896.