Domestication process of Indonesian Cemani chicken: Genetic causes for phenotypic traits

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Abstract

Understanding how phenotypes diverge and what genetic factors control phenotypic changes in domestic animals is one of the focuses when studying evolutionary biology. Researchers so far have studied genetic associations of morphological or physiological traits that contribute to diversification of domestic animals. Nevertheless, examples to find out the signature of artificial selection associated with these phenotypes are few. With development of advanced sequencing technology, complete genomes of organisms have been able to be sequenced. In addition, the development of bioinformatics has aided in the increasing ease for handling such big data sequences.

This thesis focuses on specific traits of Cemani chicken, like the fibromelanosis (Fm) phenotype, to understand the domestication process through artificial selection, and how mutations can contribute to phenotypic differences in domestic chicken. In addition, this thesis investigates genetic regions under selective sweeps and predicted candidate genes associated to Cemani traits. Moreover, I elucidate the genetic relationship between Cemani and other black chickens. Overall, this thesis provides a better understanding of the genetic basis of complex traits and the evolutionary history of domestic chicken, particularly in Indonesian *Ayam* Cemani. The specific summary from each chapter is presented below:

Chapter 2

This chapter predominantly focused on the fibromelanosis (Fm) phenotype in Indonesian *Ayam* Cemani and Chinese Silkie chicken. I proposed the evolutionary history of the Fm phenotype in Cemani and Silkie chicken by analyzing the Fm region including segmental duplications on chromosome 20 that involve the Endothelin 3 gene, *EDN3*.

Examination of the Fm region included four major components. (i) Detection of duplication boundaries of Fm chickens (Cemani and Silkie) and other domesticated chickens as control showed that duplicated boundaries were detected in Fm chickens but not wild type chicken. (ii) qPCR analysis of *EDN3* of Cemani, Silkie and other domesticated chickens and copy number variation analysis using whole sequence of duplication segment of Fm type (Cemani and Silkie) and wild type (Taiwanese) chickens concluded that Cemani and Silkie have identical genetic rearrangement of Fm phenotype due to duplication segment containing *EDN3*, indicating a single origin of the genetic cause of the Fm phenotype. (iii) Sequence analysis of 1kb of *EDN3* revealed that the duplication arose by unequal crossing-over between alleles with 0.3 MYR divergences in the ancestral Red Jungle Fowl population. (iv) Identification of selective sweeps in the Fm region (including *EDN3*) as a target region of Cemani and Silkie revealed different lengths of heterozygosity reduction in surrounding duplicated regions which suggests the region was artificially selected independently in Cemani and Silkie breeds. Furthermore, I estimated that the two breeds have diverged around 6600 ~ 9100 years ago, suggesting that the divergence of these breeds is consistent with the beginning of domestication of chicken in China.

Chapter 3

Homozygosity approach was used in this chapter to analyze a single whole genome sequence of Cemani chicken for detecting signatures of selection in the Cemani genome and identify candidate genes within these regions of putative selective sweeps. I calculated the homozygosity in every 100 kb window width of whole genome sequences of Cemani, Silkie and L2 Taiwanese (single individual each) and extracted the region with homozygosity ratio ≥0.95 (referred to as high homozygosity region, HHR). I compared HHRs among Cemani, Silkie and L2 Taiwanese and identified the genes

located within HHRs shared between the three breeds as well as in HHRs specific to Cemani chicken. I then validated the monomorphism in Cemani-specific HHRs and found that *EGFR* on chromosome 20, as well as *NT5C1A* and LOC419677 on chromosome 23 were monomorphic, indicating that these genes were under selective sweeps. This was supported by further examination in the region surrounding the genes that were also identified as monomorphic. Investigation of the function of *EGFR* revealed that the gene might have two different roles (cell pigmentation and cell growth controller), supporting that this gene may have pleiotropic effects on phenotypic traits in Cemani and commercial chickens. In addition, investigation of *NT5C1A* and LOC419677 function identified that these genes are related to fecundity traits of Kauai chickens and are positively selected in commercial chickens. Taken together, the findings in this chapter suggest that Cemani chickens are a breed of Indonesian local chickens with qualities and genetic attributes that are worthy to be developed as a commercial chicken.

Chapter 4

This chapter aimed to elucidate the origin of Cemani chicken and reveal the genetic relationship between black chickens in Indonesia (Black Kedu, Cemani and Black Sumatra), America (Black Java and Sumatra), and China (Silkie, Muchuan, Jiuyuan, Emei, Tianfu). This study used in total 60 whole genome sequence (WGS) data from 15 breeds of chickens: 10 breeds of black chickens, White Leghorn, L2 Taiwanese, Pengxian, Red and Green Jungle Fowl. Principle component analysis (PCA) using SNPs from chromosome 20 of chicken breeds revealed distinct clusters and distribution patterns of Indonesian, Chinese, and American chickens. This suggests that the different geographical distribution of Indonesian, American and Chinese chickens causes limited contact or crossbreeding between the breeds, thus limiting gene flow between the chickens and influencing genetic variation among them. In contrast with my study in

chapter 2, which revealed close relatedness between Cemani and Silkie in the Fm region, these two breeds were in a distinct cluster based on PCA on chromosome 20. Silkie was clustered together with Chinese chickens and Cemani clustered together with Indonesian chickens, indicating that selection for the Fm phenotype in Cemani and Silkie arose recently. Similarly, in BK and Cemani, these breeds shared genetic information in the Fm region but were distantly related based on microsatellite and mitochondrial DNA analysis from published studies [1,2,3]. Finally, I concluded that Cemani might be an independent breed that was brought to Kedu village and experienced interbreeding and selection with BK chickens, resulting in genetic introgression in Fm region between the two breeds.

Reference

1. Ismoyowati, Sukardi and Agus Susanto. Genetic Diversity of Kedu Chicken Based on Phenotypic Characteristics and Microsatellite Loci. International Journal of Poultry Science. 2012;11(9):605-610, ISSN 1682-8356
2. Ashari H, Sulandari S, Zein MSA, Han JL. Genetic Diversity and Relationship of Indonesian Indigenous Chickens Inferred from Microsatellite DNA Markers. Tropical Agricultural Research. 2015; 26(4): 642–654.
3. Sulandari S, Zein, MSA, Sartika T. Molecular Characterization of Indonesian Indigenous Chickens based on Mitochondrial DNA Displacement (D)-loop Sequences. HAYATI Journal of Biosciences. 2008;15(4):145-154. https://doi.org/10.4308/hjb.15.4.145