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Summary (Abstract) of Doctoral Thesis

Name in full

Anik Budhi Dharmayanthi

Title

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

インドネシアの鶏チェマニの家禽化過程：表現形質に関わる遺伝的要因について

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 chicken. 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 divergence 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 chicken in Indonesia (Black Kedu, Cemani and Black Sumatra), America (Black Java and Sumatra), and China

(Silkie, Muchuan, Jiuyuan, Emei, and 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

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Results of the doctoral thesis screening

博士論文審査結果

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Title
論文題目　Domestication process of Indonesian Cemani chicken: Genetic causes for phenotypic traits

人為選択の効果が著しく反映される動物の家畜化は、植物の栽培化と共に生物進化の研究を行うための格好の題材である。本論文で出願者は、ニワトリ、特にインドネシア原産のアヤム・セマニという品種を用い、人為選択および体内臓器と真皮における過剰メラニン産生形質 fibromelanosis（Fm）という特徴的な形質の進化について集団遺伝学・分子進化学的解析を行った。その主な結果は次のとおりである。

１）　Fm の表現型をもつ品種である烏骨鶏とアヤム・セマニには、その遺伝的要因を担うと考えられる *EDN3* 遺伝子周辺ゲノム領域の重複が見られる。この重複は、３０万年前に分化し現在でも赤色野鶏で対立遺伝子として存在する二つのハプロタイプが結合し一つのハプロタイプ上で常に二種類の遺伝子を保持することを可能にするものである。第 20 番染色体上での、この領域でのゲノム構造の類似性から烏骨鶏とアヤム・セマニの Fm は同一の突然変異に起因することが明らかとなった。また、このゲノム領域でのホモ接合度を調べることにより、この突然変異は烏骨鶏とアヤム・セマニの系統が 6600～9000 年前に分岐した後にそれぞれで独立に人為選択を受け固定したことも明らかとなった。

２）　アヤム・セマニでのヘテロ接合度を網羅的に推定しゲノムに記された人為選択・自然選択の痕跡を調べた。烏骨鶏や L2 品種（台湾産）のゲノム解析を同時に実施し比較すること、またアヤム・セマニでの単型性を確認することで、アヤム・セマニで人為選択を受けた可能性のある遺伝子として *EGFR*、*NT5C1A*、*LOC419677* 等の遺伝子を示した。*EGFR* は上皮成長因子受容体として細胞の色素沈着や細胞成長の制御に関わること、また *NT5C1A* および *LOC419677* は卵の重さや数と遺伝的に関連していることなどから、アヤム・セマニにおいてこれらの形質が人為選択の対象となってきた可能性が示唆された。

３）インドネシア、アメリカ、中国等原産の 15 系統のニワトリの第 20 番染色体の SNP に対し主成分分析を行うことで、アヤム・セマニ等黒色の色素沈着がみられる 10 品種のニワトリの遺伝的な関係を調べた。その結果これらの系統はインドネシア、アメリカ、中国等の地域毎にクラスターすることが示され、基本的には地理的に近い品種が遺伝的にも近縁であり、これら地域間での遺伝的交流が頻繁でないことが示された。これは Fm の表現型が烏骨鶏とアヤム・セマニが独立に人為選択を受けたこととも一致している。またインドネシア原産で黒色の表現型をもつ品種ケドゥ・ヒタムとアヤム・セマニには遺伝的交流があることも示された。

これらの結果は、動物の家畜化過程における人為選択の重要性を示す一例となるとともにインドネシア原産のニワトリで生じた形質の進化を語るには欠かせない知見となる。以上により、審査委員会は、本論文が学位の授与に値すると判断した。

（備考）

１．用紙の大きさは，日本工業規格（JIS）Ａ４縦型とする。

２．１行あたり 40 文字（英文の場合は 80 文字），１ページ当たり 40 行で作成する。

３．上マージン，下マージン，右マージンは２㎝，左マージンは 2.5 ㎝とする。

４．タイトルと本文の間は，１行空ける。

５．ページ番号は入れない。

６．出願者（申請者）が論文審査に合格し，博士号が授与された場合は，本紙を総合研究大学院大学リポジトリにおいて，インターネット公開する。

Note:

1. The sheets must be Japanese Industrial Standard (JIS) A4 vertical.
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