

## 学 位 論 文 の 要 旨

論文題目 Evolutionary History of *Gallus gallus* in Southeast Asia and South Pacific:  
Genetic Insights into its Phylogeography and Population Dynamics

(東南アジアおよび南太平洋における *Gallus gallus* の進化史 :  
その系統地理および個体群動態への遺伝的洞察)

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Over the past millennia, the development of agriculture in Southeast Asia, ultimately brought by human forethought and activities, was undoubtedly driven by several geological, ecological, biological, and climatic factors and cultural exchanges. The multistage processes of animal domestication exemplify how animals respond to the anthropogenic niches. Decades of research on when, where, and how domestication took place have led to a better understanding of the complex past societies, though several important questions remain unresolved.

As one of the important commensal domesticates, chickens are the most widely domesticated animal species globally. Consequently, it plays a crucial role in human societies as the largest source of animal protein and as a significant biological factor in socio-cultural development. Since domestication, chickens have been distributed throughout various countries and continents inhabited by human migration and trade expansion. This led to the evolution of subpopulations of chickens in response to natural selection pressure and selective breeding for adaptation to a variety of agro-ecological conditions and subsequently resulted in a wide range of chicken breeds today.

The course of chicken introduction in the island of Southeast Asia (ISEA) and Oceania continues to be a research area of interest, considering the rich history of human diaspora and colonization in the insular archipelago. The Philippines presents essential models for understanding Southeast Asia's evolutionary processes and species diversification. This terrestrial island faunal laboratory is considered one of the most biologically rich regions globally in animal genetic resources and offers opportunities for elucidating evolutionary and ecological processes. However, insufficient evidence links the present-day chickens to their founding lineages due to an unclear timeline of translocations and routes of dispersal across the ISEA.

To address this research gap, Chapter II extensively characterized the complete mitochondrial DNA (mtDNA) D-loop sequences of native chickens (NCs) from the Philippines and the South Pacific and Philippine red junglefowl (RJF) to assess their matrilineal phylogeny and genetic diversity and population genetic structure across ISEA and Oceania. The phylogeny reconstruction and estimation of their population genetic structure were based on 107 newly generated mtDNA complete D-loop sequences, consisting of 34

haplotypes. This study found that the Philippine chickens showed high haplotypic diversity ( $Hd=0.915 \pm 0.011$ ) across Southeast Asia and Oceania. The phylogenetic analysis and median-joining network revealed predominant maternal lineage haplogroup D classified throughout the population. Concurrently, support for the Philippine-Pacific subclade was evident, suggesting a founding lineage of the Philippine chickens before diverging to the Pacific chicken populations. This study also significantly estimated the Philippine red junglefowl at the phylogenetic tree's basal position, suggesting an earlier introduction into the Philippines, potentially from mainland Southeast Asia. The extremely low genetic differentiation and high rate of gene introgression of the Philippine chickens into the Oceanic populations suggests an expansion signal. Furthermore, this study assessed their demographic signature based on Bayesian Skyline Plot analysis and demonstrated an increase in the maternal effective population size of the Philippine chickens around 3,000-2,500 years BP. This population expansion signal likely relates to the human settlement and expansion events of the Austronesian agricultural societies in the Philippines sometime in the past.

Subsequently, the unresolved question remains whether the founding lineages of chickens introduced in the Philippines arrived as wild or descendants of wild endemic populations that potentially entered the archipelago during lowered sea levels through the Sunda shelf. Alternatively, they are descendants of domestic chickens from mainland Southeast Asia (MSEA) that have undergone feralization. To provide context to this conjecture, Chapter III characterized large-scale mtDNA sequences of chickens from MSEA (Cambodia, Laos, Thailand, and Myanmar), the Philippines, and the Pacific, spanning a geographical transect encompassing possible translocations of this species in the region. This study combined these newly generated sequence data with previously published data of ISEA chickens, Pacific chickens, and neighboring chicken populations in Asia. Furthermore, this study sought to obtain an updated perspective of the matrilineal phylogeny and demographic events that shaped the genetic diversity of SEA and the Pacific chickens.

The consensus from several molecular studies documented domestic chickens evolved from RJF somewhere in southwestern China and Southeast Asia. However, identifying their exact geographic center of origin and consequent translocation to the island archipelago has been challenging. Chapter III presented a comprehensive resolution of mitochondrial lineage diversity and phylogenetic analyses, population differentiation, and demographic inference of chickens in Southeast Asia and the Pacific region. Patterns of sequence variation indicated that chickens in the MSEA region have higher intrapopulation genetic diversity ( $Hd=0.963 \pm 0.005$ ;  $\pi=0.00782 \pm 0.00398$ ) than island populations ( $Hd=0.942 \pm 0.009$ ;  $\pi=0.00466 \pm 0.00249$ ). The substantial diversity of SEA chickens reflects the high matrilineal genetic variation documented in the major haplogroups, particularly haplogroup D with many divergent haplotypes and haplogroup V, which has been detected only in Thailand, Cambodia, and Laos. Divergent sub-haplogroups that retained ancestral mutational motifs were also observed in these lineages, likely due to the geographic proximity to the center of domestication. Strong topological supports from the phylogenetic trees consistently provide evidence for haplogroup D ancestral lineage (i.e., sub-haplogroup D2) from MSEA populations. A new matrilineage (i.e., sub-haplogroup V2) gave rise to the population of domestic chickens from Cambodia, Laos, and Thailand. Likewise, their ancestral lineage (i.e., sub-haplogroup V1) was represented in the Thai RJF (i.e., *G. g. gallus*).

Interestingly, this potential ancestral matriline sub-haplogroup D2 and newfound matriline haplogroup V were identified in sampling areas along the Lower Mekong subregion, for example, in Kampong Cham, Mondulkiri, Stung Treng, and Kratie provinces in Cambodia and Champasak and Bolikhamsai provinces in Laos. The coalescent-based Bayesian demographic analyses detected earlier effective population size expansion in MSEA chickens, while island populations showed more recent demographic growth signatures. The timing of the demographic evolution of this hypothesized founding population can be explained by the cultural importance of stock-raising in the MSEA as early as 4,000 years BP. It was well documented that agriculture and animal-raising were among the subsistence activities of domestic communities during prehistoric settlements in the broad valleys of the Lower Mekong. This study validated the unique population dynamics of Southeast Asian chickens, implying a large gene pool that has been conserved in the populations for a long time and that some were a subset of the RJF population involved in the domestication. This suggests that island chickens are potentially descendants from populations domesticated in MSEA and diverged into distinct subgroups following colonization in the island archipelago.

The earlier domestication of chickens in mainland Southeast Asia and consequent translocation to the island archipelago entering southern Philippines and Palawan are deliberately linked with human movement. However, little is known about the evolutionary links and temporal divergence between continental and island chickens, especially since archaeological records of chicken bones in the region are scarce. With the increasing genetic data and resolving power of sequence data in recent years, computer simulation methods (e.g., coalescence simulation) have been shown efficient at testing different evolutionary and demographic models of expanding and migrating populations. Furthermore, time trees or phylogenies with absolute divergence times provide incomparably richer information than a species phylogeny without a temporal clue. It makes it possible for species divergence or coalescence events to be calibrated to time.

Chapter IV estimates the lineage-specific divergence of MSEA, ISEA, and Pacific chickens using Bayesian molecular clock method. The time tree phylogeny in the coalescent framework estimates the nodal ages of biogeographically important haplogroups predominant in Southeast Asia. The coalescence time estimates of haplogroups D and V are consistent with their demographic evolution and expansion in the region, around 3,700-4,000 years BP and 3,800-4,400 years BP, respectively. Likewise, the most recent common ancestor of modern Philippine-Pacific chickens (i.e., sub-haplogroup D1b), including the ancient Pacific sequences dates to 2.1 kya (95% HPD 1,467–2,815 years). Caution is warranted for this interpretation because the coalescence age estimate of gene copies in ancestral populations is not equivalent to a population split, nor does it represent the actual onset of domestication.

In conclusion, this study provides a comprehensive insight into the genetic diversity, phylogeography, and population dynamics of Southeast Asian chickens. High-resolution matrilineal phylogeny sheds new light on the evolutionary history of globally acknowledged haplogroups of SEA and Pacific chickens. It provides evidence of a new divergent matrilineage that is distributed across its native range in the Lower Mekong subregion. This study documented the presence of a distinct island chicken subgroup that represents a unique genetic uniformity between the Philippines and the Pacific despite their geographical isolation. This latterly expanded matriline is unique to the island archipelago, suggesting a

human-mediated scenario on their translocation. Moreover, their phylogeographic signal corresponds to the initial introduction pattern of its founding matriline (i.e., sub-haplogroup D2) from MSEA. The genetic information of this valuable animal resource is essential for conservation efforts, and these data serve as a baseline for monitoring to avoid further loss of genetic diversity. Finally, this asserts excellent potential for genetic improvement and selection of traits for developing sustainable chicken production systems in Southeast Asia.

