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学 位 論 文 題 目 The mitochondrial DNA sequence analysis of
domesticated chickens and its implication
to the evolutionary origin of fighting cocks

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論文内容の要旨

This study gives clarity to the origin and evolution of domesticated chickens in Asia in view of molecular evolution. For domesticated chickens, evolution is tightly related to many aspects of human nature and culture. Because of the interest in the evolutionary process involved with such human factors, domesticated chickens have been chosen for study because of their evolutionary origin and process that has been deeply influenced by human factors.

Man has domesticated and bred chickens for various purposes for a long time. For example, a fighting cocks, *Shamo*, and long-crowing chickens, *Naganakidori*, are typical examples that must have been bred for the purposes of entertainment and appreciation that are part of human culture. For this reason, these two varieties of domesticated chickens are excellent materials for the study of how human culture has influenced the domestication and breeding process of animals.

A variety of cultural aspects of raising and keeping *Shamo* and *Naganakidori* have been observed not only in Japan but also in other Asian countries such as Thailand and China. Therefore, the understanding of the geographical origin and domestication process of *Shamo* and *Naganakidori* is one of the key issues for elucidating the relationships between human culture and its reflective breeding efforts. However, it is of prime importance to study their evolutionary origin and phylogenetic relationships using DNA sequence data, because their molecular phylogenetic relationships will be so objective and reliable as to form the bases of the discussion about domestication and breeding of chickens.

Chapter 1 explained the background of the studies of domesticated chickens from the viewpoint of evolutionary biology and cultural intervention. It also described the purpose and biological significance of the present study.

Chapter 2 focused upon the evolutionary origin of Japanese fighting cocks. A total of 42 blood samples were collected and measured three morphological traits (height, weight, and shank length) of fighting cocks, *Shamo*, in nine places in Japan. Moreover, in collaboration with colleagues in Myanmar and Southeast China, blood samples of domesticated chickens in their respective regions were also examined. Extracting the mitochondrial DNAs from the blood samples and sequencing their D-loop regions, phylogenetic trees of the domesticated chickens were constructed. The phylogenetic analysis has shown that the Japanese *Shamo* was separated into two groups: One group contained exclusively individuals from the island of Okinawa, and the other was composed of the individuals from Kyushu and Honshu islands of Japan.

The results of the phylogenetic analysis are consistent with that of the morphological analysis of the three traits. In particular, the Okinawa *Shamo* showed consistently larger values of height, weight, and shank length than the other *Shamo* groups studied. This observation supports that the Japanese *Shamo* has come from two separate origins. It is also known that different names are used for calling *Shamo* in Japan. In Okinawa, *Shamo* is pronounced as

“tautji” which is quite similar to its Chinese pronunciation. In the other parts of Japan, it is pronounced as “shamo” which is very similar to the pronunciation of the old name of “Thailand.” This cultural aspect also supports the separate origins of Japanese *Shamo*.

In Chapter 3, to elucidate the evolutionary origin and the domestication process of *Naganakidori*, nine blood samples of this variety were collected from five places in Japan. In Japan, three varieties of *Naganakidori*; Koeyoshi, Totenko, Tomaru, have been bred to develop an exceptionally long crow of over 15 seconds. The mitochondrial DNA was then extracted and its D-loop region sequenced. When a molecular phylogenetic tree was constructed, it was found that all three varieties were monophyletically related. This finding is surprisingly interesting, because the three varieties are remarkably different to each other in morphological characters. Moreover, *Naganakidori*'s group is located between the group of Okinawa *Shamo* and the other *Shamo* group. It strongly suggests that *Naganakidori* originated from *Shamo*.

Chapters 4, on the basis of the results of Chapters 2 and 3, 34 samples from 12 varieties of the ornamental chickens were examined to understand their evolutionary origin. Then, it was found that all the ornamental chickens examined have been derived from *Shamo*. It strongly suggested that all Japanese domesticated chickens including the ornamental chickens and *Naganakidori* might have been originated from *Shamo*, particularly the ancestor of Okinawa *Shamo*. The conclusion is that the intensive pressure of artificial selection was imposed on the *Shamo* population that had been already established for the tradition of cockfighting in order to create novel variation of ornamental chickens, resulting in profound differentiation of Japanese varieties of domesticated chickens.

Chapter 5 considered the background that the original *Shamo* has produced Japanese domesticated chickens, from both historical and biological approaches. The conclusion is that the ancestral *Shamo* was brought first into Okinawa and then distributed over Japan through driving forces such as appreciation, entertainment, and competition that are certainly part of human nature and culture.

Finally, it should be emphasized that this study would eventually provide basic and useful information related not only to the evolutionary process but also the important issues such as the animal protection, nature conservation, and global environments.

論文の審査結果の要旨

ニワトリは、食料のみならず、文化的要素を取り込んで趣味性の高い多様な品種が作出され現在まで保存されている。本論文では、特異的な人工選抜を受けてきた代表的な家禽であるシャモ（軍鶏）、ナガナキドリとその他の家禽品種の3者の進化遺伝学的関係を明らかにすることを目的として、これらのミトコンドリア DNA (mtDNA) の塩基配列決定と分子進化学的解析を行い、議論を展開した。

まず、闘鶏を目的に作出されたシャモの進化的起源の研究を行った。日本全国の9地点から42個体のシャモの血液を採取し、mtDNAのD-loop領域の1100bpの塩基配列を決定し、分子系統学的解析を行った。この際、2個体の赤色野鶏 (*Gallus gallus*) をアウトグループとして用いた。また、身長、体重、中足骨の長さなどの形態形質の比較解析も行った。これらの結果、日本のシャモは、沖縄シャモのみのクラスターと本州・九州シャモと沖縄シャモの混合したクラスターの2つの異なるクラスターからなることが明らかになった。さらに、「軍鶏」の呼称や古典的記述などに関する文化史的考察は、日本のシャモがこれらの2つの異なったクラスターからなること矛盾しないことを示した。

次に、ナガナキドリの起源と家禽化過程を明らかにするために、分子進化学的解析を行った。ナガナキドリは、他のニワトリの鳴き声がわずか3秒程度しか続かないのに対し、20秒近くも鳴き続けることで有名な家禽である。日本の5地域から3品種9個体のナガナキドリの血液を集め、mtDNAのD-loop領域の1100bpの塩基配列を決定した。それらの9個体とシャモを含めたその他の日本鶏74個体の合計83個体を用いて、分子系統樹を構築した。その結果、採取された地域に関係なく、形態的に大きく異なる3品種のナガナキドリのすべての個体は、分子系統樹において一つのクラスターを形成することが分かり、これらのナガナキドリは同一祖先から由来する単系統であることを明らかにした。さらに興味深いことに、ナガナキドリのクラスターは、分子系統樹において沖縄シャモのみのクラスターと本州・九州シャモと沖縄シャモの混合したクラスターとの間に位置することから、ナガナキドリはシャモを起源として作出された可能性が高いことも明らかにした。

最後に、シャモとナガナキドリ以外の12品種35個体の趣味性の高い日本の家禽の進化遺伝学的関係についてもmtDNAの配列解析を行った。その結果、調べた品種は、すべて、本州・九州シャモと沖縄シャモの混合したクラスターから由来することが明らかになった。以上の結果から、沖縄に最初もたらされたシャモが、14世紀以降に沖縄と本州・九州との交流が盛んになるにつれて、日本全国に導入され、様々な日本の家禽の品種の作出に用いられたという仮説を提示した。

これらの研究成果は、今までDNA研究がほとんど行われなかったシャモやその他の趣味性の高い日本の家禽の進化遺伝学的関係と家禽化過程を、初めて明らかにしたものである。以上のように、本論文は、分子進化学的解析に文化史的考察を加えることにより、日本における家禽の多様化過程を明らかにしており、その学術的価値は非常に高いと評価できる。

公開発表に引き続いて、学位審査委員との間で非公開の質疑応答を行った。小見山君は、いずれの質問に対しても的確な応答を行い、関連分野の学識、学力が学位を受ける水準に達していると評価した。また、本論文の内容は、すでに2編の国際誌に発表されており、また、本論文が適切な英語で書かれていることから英語の能力にも問題は無いと判断した。以上、総合的にみて本論文は、学位を受ける水準に十分に達していると結論した。