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論文題目	Effects of latitudinal difference on ecological andevolutionary characteristics of Asiatic salamanders （緯度がサンショウウオの生態・進化に与える影響）
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論文内容の要旨

Acceleration of molecular evolutionary rates (MERs) in the lower latitude regions has been documented in broad taxa, such as mammals, amphibians, plants and foraminifera. However, it is still unclear whether this correlation is general and what is the cause of this pattern. In the present study, I investigate evolutionary history of diversification of the eastern Japanese salamander genus *Hynobius* (Caudata: Hynobiidae), and I provide an example showing a negative correlation between latitude and MERs. In addition, I estimate the cause of this latitudinal pattern based on ecological and physiological studies.

In the first part of the thesis, phylogenetic relationships among the *Hynobius* species were estimated by molecular phylogenetic analyses of mitochondrial cytochrome b (Cyt b) and cytochrome c oxidase subunit I (COI) genes. The *Hynobius* salamanders are distributed from the Eastern Asia to Central Asia. The Japanese Islands have the highest species diversity of *Hynobius*, and 57% of the 23 species recorded on the Japanese Islands are endemic to the islands. Thus, the Japanese *Hynobius* salamanders are excellent model systems to investigate how species diversity has been originated. The inferred phylogeny suggests that the northernmost lineage occurred from Hokkaido is first diversified from other *Hynobius*. In the next, lineages of the Eastern and Western Japan, and those on Taiwan were separated. In addition, the lineages of the Western Japan included species phylogenetically closer to the species of the Asian Continent than those of the Japanese Islands, suggesting repeated immigration of the species among the Asian Continent and the Japanese Islands. The history of diversification in the Japanese *Hynobius* most likely reflects geological history of the Japanese Islands, repeated isolation and connection of the islands from the continents. The present findings show how biological diversity has been originated in the Japanese Islands.

In the second part of the thesis, I focus on how generation time differs among the regions of different latitude. It is still unclear how generation time of *Hynobius* salamander changes due to the difference in climate. As salamander has unique lifestyle and life history, it is an excellent model to investigate how generation time changes by climatic change. Age structure of a breeding population of *Hynobius lichenatus* in Sendai (140°47'E, 38°14'N) was examined to estimate the generation time. The patterns of lines of arrested growth on the cross-sectional bones (LAG) were examined to estimate the age structure of breeding adult salamanders and their maturation ages. The age for each sex was estimated using 27 individuals. In the breeding population of *H. lichenatus* from Sendai, no significant difference was detected in both of the age structure and the maturation age between males and females. The present results were compared with previous study intended for age structures on two breeding population/metapopulation of *Hynobius tokyoensis*. Significant differences in age structure were found between the Chiba and Sendai populations. The population from Chiba becomes to mature earlier and has a shorter life than that from Sendai. In contrast, there is no difference in age structure between the populations from Tokyo and Sendai. Present findings suggest that generation time is not necessarily affected by temperature but is affected more strongly by other environmental or ecological factors.

In the third part of the thesis, hypothesis of latitudinal gradient of MERs was tested via phylogenetic analyses of mitochondrial Cyt b and COI genes on the eastern lineages of the Japanese *Hynobius* salamanders that occurred from the range of 34°N - 44°N. Significantly higher MERs were detected in the lower latitude regions than in the higher latitude regions. Neutrality of each gene showed latitudinal decline toward lower

latitude for COI, whereas no significant difference in neutrality among different latitude regions was detected for Cyt b. It is unlikely that population size causes this phenomenon, because population size should affect both of these gene regions. Latitudinal difference in pressure of natural selection was detected in COI gene region, but the direction of intensity of selection pressure was opposite to the direction of MERs. Thus, there is no evidence that natural selection results in this latitudinal pattern. It is most likely that the observed latitudinal trend is a result of enhanced mutation rates in the populations of lower latitude. Metabolic activity is expected to affect mutation rates via harming the DNA with free radical oxygen, thereby organisms with long hibernation is supposed to have lower evolutionary rates. Latitudinal gradient of the length of dormant season and generation time were examined to test the above hypothesis. The relationship between latitude and length of active phase can be estimated by examining relationship between the date of first oviposition occurred and latitude of the locality where the oviposition was recorded, because their mating seasons come after the hibernation season. The analyses showed that the active phase is longer in the regions of lower latitude. Thus, metabolic rate of salamander is expected to be higher in warmer environments, suggesting increase of molecular evolutionary rate in the populations of lower latitude.

In conclusions, inferred phylogenetic relationships among *Hynobius* species reveal complex history of isolation and immigration of species between the islands of Japan and the Asia Continent. Based on the analyses of age structure, generation time is not necessarily affected by temperature or latitude in *Hynobius* salamanders. Acceleration of MERs in lower latitude regions was detected in *Hynobius* species. Latitudinal difference in metabolic activity is the only factor which can affect the latitudinal trend in MER in *Hynobius*. It is unlikely that generation time results in the latitudinal pattern of MER in *Hynobius*. Although further comprehensive analyses would be needed, the present study strongly suggests that climatic condition affect mutation rate through the change of activity and metabolic rates.

論文審査結果の要旨

本博士論文は、日本および中国、台湾、朝鮮半島の *Hynobius* 属サンショウウオを材料として、①日本産本属の種群における遺伝的分化および生物地理パターンの形成プロセス、②性成熟に要する期間の緯度勾配、③ミトコンドリア DNA の進化速度の緯度勾配、およびそれに関与する機構について明らかにした。

(1) まず、*Hynobius* 属サンショウウオのミトコンドリア DNA (COI 遺伝子領域および Cyt b 遺伝子領域) の塩基配列を解析し、アジアにおける本属各種の系統関係を推定した。さらに地史との比較から、これらの種が分化してきた進化史を解明した。その結果、2000 万年前にアジア大陸から分化したのち、大陸との再度の移住、分化を経て、現在の文化が生じたと考えられた。また現在の日本における種の分布は、700 万年前にはほぼ成立していたと考えられた。

(2) 次に、骨の年輪分析から性成熟に達する年齢と、繁殖個体の齢構成を推定し、関東地方と東北地方の個体群を比較することによって、成熟に要する期間に緯度勾配が認められるかどうかを調べた。従来の研究では、低緯度ほど早く成熟し、世代時間が短くなるとされていたが、本研究ではこのような緯度勾配は認められなかった。むしろ関東地方の 2 地点で大きく成熟齢が異なっており、こうした生活史形質の変異は、緯度とは別の要因によって決まっている可能性が高いことがわかった。

(3) 東北地方から関東地方の *Hynobius* 属サンショウウオ個体群について、ミトコンドリア DNA の分子進化速度を求め、系統樹上でベイズ法により集団間で速度の比較を行った。その結果、いずれの遺伝子領域においても低緯度ほど進化速度が有意に速くなるという結果が得られた。しかし、従来の研究で主張されている有効集団サイズや中立性の緯度勾配からはこの進化速度の緯度勾配を説明することはできなかった。また(2)の結果から、生活史の違いもこのパターンに関与していないことがわかった。一方、冬眠に要する期間から推定された総代謝量は、緯度勾配を示しており、高い代謝量が分子進化速度を高めるとする仮説を支持するものとなった。

以上のように本論文では、サンショウウオにおいて分子進化速度に緯度勾配があることを明らかにし、分子進化速度が代謝量に影響されて緯度勾配を生じるとする仮説を検証した初めての報告である。

このように重要かつ新しい発見を多く含む点から、本論文は博士論文として適当であると考えられる。また上記(2)については、それぞれすでに国際誌に出版済みであり、また(1)(3)についても投稿予定である。以上の点は山本朋範君が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。従って山本朋範君提出の博士論文は、博士(理学)の学位論文として合格と認める。