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学 位 論 文 題 目　　Molecular phylogeny and evolution of prosimians based
　　　　　　　　　　on complete sequences of mitochondrial DNAs

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Primate evolution draws special attention because of its direct relevance to the human origins. Particularly, prosimians (tarsiers and strepsirrhini) are the first diverged species among primates and have a close relation with the primate origin. In addition, the adaptive radiation among prosimians of Madagascar provides an excellent model for studies of evolutionary diversification. The phylogenetic relationships and divergence times of primates have been of special interest to anthropologists and evolutionary biologists.

Chapter 1 presents the classification and the features of primates, especially prosimians.

In my study, complete mitochondrial DNA (mtDNA) sequences of primates were used. I review the nature and characteristics of mtDNA in chapter 2.

It is sometimes the most difficult step in such studies to get samples particularly from endangered species. In chapter 3, I show a successful amplification and sequencing of mt-genome of *Propithecus* (sifaka) from feces sample, using the extract method of Chelex-100 Phenol-Chloroform or QIAamp DNA Stool kit (Qiagen) in combination with FTA cards (Whatmann). For biologists, such a noninvasive sampling method should be an important resource that will provide greater opportunities to collect and to use invaluable samples. By using the mt-genome sequence of sifaka obtained by this work with other published sequences of primates, I estimated the phylogeny of primates, and demonstrated that the evolutionary rate acceleration, particularly in the amino acid level, occurred in the Anthropoids lineage after they diverged from tarsier.

Major lineages among anthropoidea are well represented by complete mtDNA

sequence data, but only one complete mtDNA sequence from a representative of each of the infraorders in prosimians (*Nycticebus coucang* (lorisiformes), *Lemur catta* (lemuriformes), *Tarsius bancanus* (tarsiiformes)) has been described. So, I determined new complete mtDNA sequences from 6 lemurs (including the sifaka described in chapter 3), 5 lorises and one platyrrhini, and combined the data set with the 14 primates sequences reported to the data base in order to carry out an extended study of the prosimian relationships among primates. In chapter 4, I present the first systematic analyses using abundant complete mt genome data derived from 11 prosimians; representatives of three families out of five of lemuriformes, the Asian lorisidae (south Asia, south-east Asia) and the African lorisidae and galagidae from lorisiformes, and tarsiiformes. The purposes of this study are (1) to clarify problematic relationships among prosimians based on mtDNA data and (2) to investigate the advantage of mtDNA data in studying the phylogenetics of primates. The position of tarsiers among primates and the question of the lorisidae monophyly could not be resolved by the maximum likelihood (ML) and neibor-joining (NJ) analyses with several data sets. The KH and SH tests indicated that the differences between several alternative trees are not significant. As to the position of tarsiers, three alternative topologies (the monophyly of haplorrhini, the monophyly of prosimians, and tarsiers being the basal position in primates) were not rejected at the significance level of 5%, neither at the nucleotide nor at the amino acid level. As to lorisiformes, three distinct lineages (African lorisidae (potto, *Perodicticus*), Asian lorisidae (*Loris* and *Nycticebus*), and monophyly galagidae (*Galago* and *Otolemur*)) were detected as well. In addition, the significant variations of C and T compositions were observed across primates. These variations of base composition could sort primates to three groups. The first group is catarrhini, higher

primates involving human, having a high percentage of C. The second group consists of platyrrhini, tarsiiformes, and lemuriformes having a low percentage of C. The third group is lorisiformes having an intermediate percentage of C between the above 2 groups. These variations of base composition across primates were found some significant correlation to codon and amino acid bias and they might affect the phylogenetic analyses. Furthermore, I used AGY data sets for phylogenetic analyses in order to retain information from transitions between purines and to remove the effect of transition between pyrimidines. As to the analyses of protein-encoding region, the support value of the position of tarsiers branching off first among primates were decreased from 85% to 60% with the HKY + Γ model, from 88% to 57% with the GTR + Γ model using data excluding third codon positions and decreased as well using all codon position data. The rRNA data sets yielded the topology, with tarsier and the anthropoids forming a monophyletic group where bootstrap support increased from 34% to 57% with the HKY + Γ model and from 36% to 60% with the GTR + Γ model. In this study, the ML analyses could not give a fully resolved and reasonable inference about the problematic taxa, that is, tarsiers and potto. The analyses of AGY data sets, however, provided a medium support for the monophyly of haplorhini. I feel that the monophyly of haplorhini might be screened by the variation in base composition of mtDNA across species.

Although the phylogenetic relationships of living primate species are relatively well established, the divergence times of living primates estimated by molecular data and the biogeographic history of primates are still controversial. Furthermore, the estimation of the divergence date of lemuriformes-lorisiformes and the adaptive radiation among lemurs endemic to Madagascar was still problematic due to the lack of

terrestrial fossils from the Tertiary of Madagascar. In chapter 5, I estimate and discuss the divergence dates among primates species. To estimates the speciation dates within primates, particularly within strepsirrhini, I used the new mt genome sequence data from 12 primates together with those from 14 primates and 26 nonprimate mammals available in the public databases. I used amino acid sequences of mtDNA for estimating divergence times of distantly related species and employed a Bayesian method (Thorne et al. 1998, Thorne and Kishino 2002). The Bayesian approach does not assume a uniform clock and does not require prior specification of rates for branches and permits the incorporation of multiple constraints from the fossil record. Seven calibration points, including one calibration within the primate clade, were used based on paleontological data. Divergence ages were estimated in this study for the following crown groups: 33.1 ± 3.7 (26.2-40.8) million years ago (mya) for lorisidae, 20.6 ± 3.1 (23.4-39.2) mya for galagidae, 36.5 ± 3.8 (29.3-44.4) mya for lorisiformes, 26.2 ± 3.3 (20.2-33.0) mya for lemuridae, 55.6 ± 3.8 (48.1-63.2) mya for lemuriformes, 64.5 ± 3.6 (57.5-71.7) mya for strepsirrhini, 70.2 ± 3.4 (63.5-77.2) mya for haplorrhini. and 76.0 ± 3.3 (69.5-82.7) mya for primates. The lorisiformes diverged 36.5 ± 3.8 (29.3-44.4) mya into Galagidae and Lorisidae which is well in agreement with the recently discovered fossils by Seiffert et al. (2003) from the late Middle Eocene, which suggested that the basal divergence between extant Galagidae and Lorisidae was under way by at least 38-40 mya.

In chapter 6, I reexamined the biogeographic scenarios proposed for the origin of strepsirrhini (lemuriformes and lorisiformes) and dispersal of the lemuriformes and lorisiformes with the data obtained by this study, as well as the fossil record and the geological history of the relevant geographic areas. The enigmatic questions in strepsirrhine evolution are when and how lemurs first arrived in Madagascar, when and

how lorises spread over Asia and Africa. By using the correlations between divergence age and geological conditions, I hoped to gain a better understanding of the speciation scenarios of lemuriformes and lorisiformes. The extant strepsirrhini colonize Africa, Asia, and Madagascar. Where is the origin of strepsirrhini? In this study, two hypotheses arise about dispersal and migration of strepsirrhini. One hypothesis is that strepsirrhines originated in Africa and that Madagascar and Asia were colonized by respective single immigration events. In agreement with paleocontinental data, the molecular analyses suggest a crossing of the Mozambique Channel by rafting or hopping island between the late Cretaceous and the middle Eocene, whereas Asia was most likely colonized between the early Eocene and the middle Oligocene on a continental route. Combining the colonization theories, it seems likely that the initial separation between lemuriformes and lorisiformes occurred in Africa, followed by a monophylic lemuriformes progenitor invading Madagascar. In Africa, the lorisiformes subsequently underwent two major splitting events, with a first one separating galagidae and lorisidae and a second one leading to two lorisidae lineages, of which one migrated to Asia. Another hypothesis is that that strepsirrhini originally inhabited Indo-Madagascar, rather than Africa, and that lemurs became isolated when Madagascar separated from India, on which the ancestral lorisiformes evolved. Subsequently, lorises could have migrated to Africa after India collided with Asia, reaching Africa during the Eocene. The Indo-Madagascar continent split from the African mainland and reached its current position 400 km east of Africa 121 mya. Later, 88 mya, the Indian subcontinent split from Madagascar, drifting north-eastward and colliding with Asia 56-66 mya. A later time of breakup between the Indian and Malagasy or an earlier divergence of strepsirrhini might be compatible with this Indo-Madagascar origin hypothesis.

Bugtilemur fossil clearly enhances the critical role of the Indian subcontinent in the early diversification of lemurs and constrains paleobiogeographic models of strepsirrhine lemur evolution (Marivaux et al. 2001). However, this fossil suggested another interpretation that *Bugtilemur* might alternatively be interpreted as a very specialized adapiforms (Marivaux et al. 2006) and is needed more discussion. A similar scenario (adapted from molecular data) has been suggested for the Indian frog (Biju and Bossuyt 2003) and the ratites, large flightless birds (Cooper et al. 2001).

論文の審査結果の要旨

　霊長目の進化は、ヒトの起源を考察する上でも大変重要である。特に、霊長目のなかで最も古くに分岐したと考えられる原猿類の系統進化を明らかにすることが、霊長目の起源を考察する上で鍵をにぎると考えられる。しかしながら、原猿類の系統進化については、不明瞭な点や研究者間で矛盾する結果も報告されている。特に、メガネザル下目の系統関係は不明瞭である。そこで、本論文では、原猿類の系統進化を分子レベルで明らかにすることを目的として科レベルや生息地域でそれぞれの代表種となる原猿類のミトコンドリアDNAの全塩基配列を決定し、それらを用いた分子進化学的な研究を行っている。

　論文は6章からなり、第1章では、霊長目、特に原猿類の特徴について記載してあり、第2章ではミトコンドリアDNAの特徴について明確に解説してある。第3章では、入手が困難である原猿類のミトコンドリアDNAを得るための方法について説明してある。具体的には、マダガスカル島において、原猿類の糞サンプルを入手し、筆者が条件検討を繰り返し考案したChelex-100を用いた方法でDNAを抽出し、そのDNAを鋳型として2回のPCRを行うことでミトコンドリアDNAの部分塩基配列を決定している。その実験を繰り返すことでミトコンドリアのほぼ全長をカバーする精度の高いDNA塩基配列を得ることに成功した。第4章および第5章では、本論文において決定した塩基配列を含む各種ミトコンドリアDNA配列を用いて詳細な分子進化学的系統解析を行い、以下に述べる結果を得ている。1)最尤法を用いて原猿類の系統関係を推定した結果、ロリス下目、キツネザル下目はそれぞれ単系統になることが明らかになった。さらに、真猿類、メガネザル下目、曲鼻猿類 (ロリス下目、キツネザル下目)の3グループの系統関係と、ロリス下目内部の関係を解析した結果、可能な315通りのトポロジーから9通りの可能性に絞り込むことができたが、それぞれの可能性を有意に棄却することはできずに最適なトポロジーを決定することはできなかった。2) 系統関係を明確に判定できない理由として、霊長類全般にわたってミトコンドリアDNAの塩基組成中のCとTのバリエーションが大きいことが挙げられ、特にヒトを含む狭鼻猿類(Catarrhini)と比較して、キツネザル下目、メガネザル下目、新世界ザルのミトコンドリアDNAの塩基組成中にCの減少、Tの増大が見られることを見いだした。 3) そこで、CとTを同列にピリミジン(Y)として扱うAGY法を用いて、再度系統関係を解析した結果、真猿類とメガネザルが近縁関係になることを支持するブートストラップ値が上昇した。4) 分子時計一定を仮定しないで、枝ごとに進化速度一定を仮定するThorneらによるベイズ法を用いて霊長目の分岐年代の推定を試み、現生霊長目の種分化を約7600万年前と推定した。また、メガネザルの種分化を約7000万年前、キツネザル類、ロリス類の種分化をそれぞれ、5600万年前、3700万年前と推定できた。特に、ロリス類の分岐年代については、最近報告された化石のデータともよく合致し信頼性の高い分子レベルの結果であると言える。さらに、新世界猿、旧世界猿の分岐年代もそれぞれ、4500万年前、3000万年前と推定することができた。以上第4章おいび第5章で得られた分子系統解析の結果をもとに第6章では、原猿類の現在の生息分布に至るまでのシナリオについて考察している。特に、これまで有力と考えられていた曲鼻猿類のアフリカ起源説に加えて、インド・マダガスカル起源説を新しく提唱している点は大変興味深い。

　以上の論文は、マダガスカル島における原猿類の糞サンプリング、糞サンプルからのDNA

抽出条件の検討、塩基配列の決定、そして得られたミトコンドリア DNA 塩基配列を用いた分子進化学的解析と大変多岐にわたる研究成果である。特に分子進化学的解析から、原猿類の系統関係と、原猿類を含む霊長目の分岐年代を推定した点は、高い学術的価値を持っている。さらに、分岐年代の推定と化石証拠を照合し原猿類の現在の生息分布にいたるまでのシナリオを考察している点など独創的かつ学際的研究として高く評価できる。