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論　文　名	「Molecular Phylogeny of Subgenus *Ceratotropis*, Genus *Vigna* (Leguminosae) Based on cpDNA Non-coding Region Sequence Analysis（葉緑体 DNA 非コード領域の塩基配列解析に基づくササゲ属 *Ceratotropi*s 亜属の分子系統）」		
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論文要旨

Knowledge of phylogenetic relationships provides insights into the origin and evolution of cultivated plants which are important for the human society. The subgenus *Ceratotropis* (Piper) Verdcourt of the genus *Vigna* (Leguminosae) includes five important legumes, mung bean (*Vigna radiata*), black gram (*V. mungo*), moth bean (*V. aconitifolia*), azuki bean (*V. angularis*) and rice bean (*V. umbellata*) for food, and other species utilized as forage and cover crops. The wild species of *Ceratotropis* inhabit coastal sandy soil, lime stone hills, rocky places, forest margins, roadsides and open fields in the southeastern part of Eurasia. Despite the agronomical and ecological importance of the subgenus, little is known about the genetic diversity and interspecific relationships among the domesticated and wild species of *Ceratotropis*. This study aimed to investigate the intra- and inter-specific relationships in subgenus *Ceratotropis* in order to undserstand its origin and evolution for better utilization of genetic resources. Chapter I reviewed conventional taxonomic, geobotanic and phylogenetic studies in *Ceratotropis*. Chapter II described characterization of *Vigna* species from Myanmar at molecular level. Chapter III mentioned the molecular phylogeny of subgenus *Ceratotropis* based on four chloroplast DNA region sequences. Chapter IV examined intraspecific diversity of wild, weedy and cultivated azuki beans for discussing the origin of crops. Chapter V included general conclusions.

In the chapter I, taxonomy, diversity and ethnobotany in subgenus *Ceratotropis* were reviewed. The subgenus consists of ca. 21 species which are distributed in tropical South Asia, Himalayan highlands, subtropical South East Asia, and temperate East Asia. The subgenus is considered to be a morphologically homogeneous group, however it is subdivided into two morphological groups (Azuki bean group *s. str.* and Mung bean group) or three sections (*Angulares*, *Ceratotropis* and *Aconitifoliae*) on the basis of seedling characters. Based on cross compatibility, three or more isolated gene pools were proposed in *Ceratotropis*. Although inter- and intra-specific relationships within the *Ceratotropis* have been investigated by the morphological, biochemical, isozyme and DNA analyses, there still remains little understanding of phylogeny. Morphological and molecular studies indicated incongruency on relationships among species and subdivision of subgenus *Ceratotropis*. These different opinions on phylogenetic relationships and diversity of subgenus *Ceratotropis* were mainly based on limited samples from South Asia and East Asia. Furthermore, different geographic regions have been proposed on the cradle of domesticated species. For example, there are two hypotheses on the origin of domesticated azuki bean (*V. angularis* var. *angularis*): East Asian origin vs. multiple origins, although direct progenitor of cultivated azuki bean is unclear.

In the chapter II, phylogenetic placement of *Vigna* species from Myanmar were determined using three non-coding region sequences of *trn*T-F. Fifteen species including author's collections in field surveys and those from National Seed Bank of Myanmar were selected. Phylogenetic tree based on 110 informative characters in *trn*T-F divided 10 Myanmar species into two well differentiated groups, the azuki bean group and the mung bean group. Six Myanmar species were clustered with the azuki bean group and four species with the mung bean group. No lineage differentiation was found among three races of *V. angularis* and *V. nepalensis*. Wild *Vigna* accession from Myanmar showed similar morphological features to wild azuki bean and shared a 51-bp deletion with *V. angularis* and *V. nepalensis*. Three *V. minima* accessions grouped as a distinct clade. *Vigna riukiuensis* nested with *V. nakashimae* and sistered to *V. hirtella*, *V. umbellata,* and *V. exilis*. The mung bean group radiated into five subgroups. *Vigna trinervia* from Myanmar clustered with tetraploid *V. reflexo-pilosa*. A high level of difference in substitutions, indels and microsatellites in the *trn*T-F region was found between mung bean and black gram. Phylogenetic relationships and diversification pattern suggest that Myanmar is an overlapping area of two genetically distinct groups.

In the chapter III, phylogenetic relationships and histotrical biogeography of subgenus *Ceratotropis* were investigated by sequencing the multiple chloroplast DNA regions (*pet*A-*psb*J, *psb*D-*trn*T, *trn*T-*trn*E and *trn*T-*trn*L) in 42 accessions of 18 species and four outgroups. The maximum parsimony and Bayesian phylogenetic trees based on 224 parsimony informative sites revealed robust interspecific relationships and presence of two geographical groups. The East Asia-South East Asian clade included two domesticated and 9 wild species and was subdivided into two subgroups, temperate and subtropical groups. Temperate group included *V. minima* from Myanmar, wild, weedy and cultivated *V. angularis* and *V. nepalensis,* and subtropical group comprised *V. nakashimae*, *V. riukiuensis*, *V. minima* from Indonesia, *V. hirtella*, *V. exilis*, wild and cultivated *V. umbellata* and *V. tenuicaulis*. The Indian subcontinent clade included four domesticated and eight wild species in tropical regions of South Asia. This clade, tropical group, was divided into two subgroups: *V. trinervia*- *V. reflexo-pilosa*- *V. trilobata* subgroup and *V.* mungo- *V. grandiflora* subgroup. Character mapping on the phylogenetic trees indicated multiple evolution of hypogeal germination and pod pubescence in subgenus *Ceratotropis*. Applying the absolute synonymous substitution rate of ~1.3 x 10^{-9} substitution/site/year and assuming $T = D_A/2\lambda$, the divergence of Indian subcontinent clade from East Asia-South East Asia clade was estimated at 4.2 mya (D_A = 0.01116). Temperate group diverged from subtropical group at 1.9 mya (D_A = 0.0494) within East Asia- South East Asian clade. Diversification of subgenus *Ceratotropis* into different geographical and ecological groups might be in Pleistocene-Holocene epoch.

In the chapter IV, in order to investigate the sequence variation in domesticated azuki beans, four chloroplast intergenic spacer regions (*trn*Q-*rps*16, *trn*D-*trn*Y, *trn*T-*trn*E, *psb*D-*trn*T) were analyzed in 31 accessions representing wild, weedy, cultivated azuki beans and related *V. nepalensis*. Wild azuki bean, *V. angularis* var. *nipponensis,* from East Asia shared the same substitution and microsatellite repeats with weedy and cultivated azuki beans. Himalayan endemic species, *V. nepalensis*, was closely related to *V. angularis* suggesting *V. nepalensis* to be one of the representatives of wild azuki bean adapted to relatively warm climatic conditions of Himalayan highlands. Intraspecific chloroplast DNA phylogeny and haplotype network revealed two geographical groups of wild azuki beans. Shared synapomorphy, microsatellite variation, and no lineage differentiation between Himalayan and East Asian cultivated azuki beans suggest that the maternal genome donor of cultivated azuki bean is the wild populations from East Asia. The cultivated azuki bean might disperse throughout Asia after the domestication in Far East Asia.

Present study provides the accurate phylogenetic information on species relationships, evolutionary pathways and origin of subgenus *Ceratotropis*. Chloroplast non-coding region sequences indicated Pleistocene-Holocene diversification of subgenus *Ceratotropis* into two major groups in Indian subcontinent and South East Asia. Closest wild relatives of domesticated species of *Ceratotropis* were revealed in different eco-geographic groups. Presence of only two major groups and multiple origin of hypogeal germination type disagree with the three sections in subgenus *Ceratotropis*.

Chloroplast haplotype network of three races of *V. angularis* suggests domestication of azuki bean in East Asia. Ecological and geographic factors assumed to play an important role in the diversification of subgenus *Ceratotropis*. The results of present study will provide a foundation for sustainable utilization of wild species as genetic resources in gene transfer and crop improvement as well as *in situ* and *ex situ* conservation of plant diversity.

審査結果の要旨

ササゲ属 *Ceratotropis* 亜属は，リョクトウ *Vigna radiata*，ケツルアズキ *V. mungo*，モスビーン *V. aconitifolia*，タケアズキ *V. umbellata*，アズキ *V. angularis* などの食用豆を含み，野生種でも種子や植物体が無毒なため，広汎に経済利用できる植物群である。ユーラシア東南部に分布する野生種は，資源植物として直接利用できるだけでなく，主要な食用豆の品種改良における遺伝資源として極めて有用である。これらの戦略的活用には，その遺伝的多様性の成立過程を十分に把握しておく必要がある。本研究は，実態が知られていなかったミャンマー産種の遺伝的多様性，亜属全体の系統進化およびアズキと祖先野生種における種内分化を葉緑体ＤＮＡの塩基配列情報解析によって分析し，資源植物および遺伝資源としての野生種の活用について考察している。

本論文では，本亜属と近縁属について系統分類学上の知見を総説した後，まず，ミャンマー産種を含む 15 種 35 系統を用いて *trn*T-F の非コード領域の塩基配列 2,003 bp を決定し，分子系統樹(CI=0.75, RI=0.91)を構築した。ミャンマー産 10 種は，リョクトウ類とアズキ類の２群の系統内クレードに分かれて所属し，高い多様性を示した。系統樹のなかで複数の野生アズキと *Vigna nepalensis* は明瞭に分化せず，一つのクレードを形成し，これらと同じ 51-bp の欠失をもつミャンマー産の野生アズキ類似種も同じクレードに所属した。広域分布種ヒメツルアズキ *V. nakashimae* と先島の固有種ヒナアズキ *V. riukiuensis* も一つのクレードを作り，その近縁性が認められた。また，ミャンマー産の２倍体種 *V. ternervia* は，４倍体種オオヤブツルアズキ *V. reflexo-pilosa* と同じ系統関係を示し，オオヤブツルアズキの祖先種と推定された。インド亜大陸とアジアとに接するミャンマーは本亜属の多様性のホットスポットとなっていた。

次に，本亜属における系統分化と地史的関係を検証するために，18 種 42 系統と４種の外群を用い，４つの遺伝子間非コード領域を分析した。全長 3,635 bp の塩基配列情報における 224 ヶ所の有効サイトに基づいて形成された分子系統樹(CI=0.809, RI=0.901)は再現性の高いクレードから構成され，本亜属の系統分化と系統群の分岐年代と形質進化を推定できた。完全な単系統性を示す本亜属は，420 万年前にインド亜大陸と東南アジアに対応したリョクトウ類とアズキ類の大きな二つの群に分岐した後，アズキ類では 190 万年前に温帯群が亜熱帯アジア群から分岐して，それぞれにおいて現在の種に相当する種分化が進んだと推定された。この結果は，種子の発芽形態や托葉の形態や有毛性などの形態情報を重視

していた従来の節や種群の分類様式を否定するものである。

本亜属における栽培種の歴史的成立を考証するために，アズキと野生アズキ *V. angularis* var. *nipponensis* の31系統および2系統の *V. nepalensis* を用いて4種類の遺伝子間領域を分析した。2,076 bp の塩基配列情報に基づく分子系統樹およびハプロタイプ・ネットワークは，単系統の群内に2つの小グループの存在を示し，東南アジアからヒマラヤ高地の温帯林に野生する群と極東アジアに生育する群を明示した。栽培アズキは，ヒマラヤから日本までの広い地域で栽培される材料を分析したにも関わらず，極東アジアの野生アズキと同じ変異を示し，多様性の程度も低かった。この結果は，野生アズキが温帯群として亜熱帯群より分化した後，東南アジアの高地において種分化し，照葉樹林の拡大とともにアジアに広く野生分布し，極東アジアに伝播した野生アズキから栽培化した栽培アズキが利用民族の移動とともにアジアに広く広がった過程を反映していると推定された。

本論文の成果は，本亜属における歴史的多様化の過程の解明に基づいて遺伝資源活用や保全における生物多様性の意義を合理的に説明しており，遺伝資源学における基礎的知見を与えるだけでなく，資源植物学，育種学，分子分類学など関連領域の学術に広く貢献すると考えられる。したがって，最終試験の結果と併せて，博士（応用生命科学）の学位を授与することを適当と認める。