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学 位 論 文 題 目 Molecular phylogenetic analysis of the  
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(博士論文の要旨)

Molecular phylogenetic analysis of the urophyllum group in the genus *Fagopyrum* by the maximum likelihood method

Molecular phylogenetic analyses using multiple sequence data were performed by maximum likelihood (ML) methods for attempts to reach a consensus on tree topology in order to reconstruct a reliable tree and to estimate divergence dates for the urophyllum group in the genus *Fagopyrum* (Polygonaceae). In Chapter 1, nucleotide sequences of *rbcL-accD*, *trnK* intron, and *trnC-rpoB* spacer region in chloroplast DNA (cpDNA) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) available from GenBank database were re-examined for phylogenetic inference and tree topologies estimated from these different genes were compared. A statistical analysis by the Kishino-Hasegawa (KH) test indicated that the tree topologies might be different between the nuclear and chloroplast DNA phylogenies, although insufficient taxon sampling in the nrDNA ITS sequence data did not allow me to decide whether the topological incongruence is real. Topological incongruence among gene trees may include important issues on evolution, and therefore confirmation by the other evidence is needed. For this purpose, in Chapter 2, two novel nuclear genes: *FLORICAULA/LEAFY* (*FLO/LFY*) and *AGAMOUS* (*AG*) were isolated from all the *Fagopyrum* species. The *FLO/LFY* and *AG* sequences turned out to be phylogenetically more informative at the intrageneric level than the cpDNA sequences. Congruence among these gene trees, inferred by the KH-test, demonstrated that topologies were partially incongruent between the nuclear and chloroplast DNA phylogenies. The nuclear DNA sequence data supported a monophyletic relation of *F. stative*, *F. leptopodum*, *F. gilesii*, and *F. jinshaense*, whereas the former two species formed another monophyletic relation with the *F. capillatum*-*F. gracilipes*-*F. gracilipedoides*-*F. rubifolium* clade excluding *F. jinshaense* in the synthetic cpDNA phylogeny. In addition, two divergent sequences of *FLO/LFY* were found in *F. rubifolium* (tetraploid). One of these was sister to *F. gracilipedoides* and another was sister to *F. stative*, and a monophyletic relation of these two genes was rejected by a bootstrap analysis. These results suggest that hybridization may have occurred during diversification of *Fagopyrum* species in the urophyllum group, and that *F. rubifolium* is possibly allotetraploid species. In Chapter 3 and Chapter 4, molecular dating was performed in order to have another insight into *Fagopyrum* evolution. Because conflict over time scale has been explained by potential sources of error and bias in estimations based on molecular clock methods, these factors that affect dating should be considered accurately. In Chapter 3, I performed date estimation by a Bayesian approach that can evaluate heterogeneity in the evolutionary rate among lineages in the framework of land plants, using fossil data to yield constraints on some node times. Dates estimated from different genes (*atpB*, *rbcL*, and 18S rDNA) were fairly consistent but they were sensitive to the number of calibrations involved. The best estimate derived from *rbcL* calibrated with five fossil dates showed a reasonable time scale for angiosperm evolution, suggesting that the improved technique of molecular date estimation may provide evolutionary implications, especially for lineages for which sufficient fossil information is not available, such as *Fagopyrum*. The date for divergence between *Polygonum* and *Fagopyrum* was estimated to be 30 MYA. The date for separation between the two *Fagopyrum* subgroups was estimated to be 20 MYA. In Chapter 4, using the 20 MYA date for the *Fagopyrum* subgroup separation as a reference, divergence dates among *Fagopyrum* species were estimated with *FLO/LFY* and *AG* by the ML method that assumed local molecular clocks. Divergence dates of each *Fagopyrum* subgroup were estimated to be 9.6 MYA for the cymosum group and 8.2 MYA for the urophyllum group.

The primary object of this thesis is to reconstruct phylogenetic trees, in which various phylogenetic and evolutionary questions should be addressed by using multiple sequence data. In alternative methods of maximum parsimony (MP), neighbor joining (NJ), and maximum likelihood (ML), the ML method is the most helpful to deal with a number of sequence data for

investigating topological differences among the gene trees and for synthesizing these results into total evidence. By comparing likelihood scores, the ML method provides comprehensive ways for testing specific hypotheses. Therefore, I mainly used the ML method in order to elucidate hypothetical issues on topological incongruence. The second object of my study is to estimate divergence date of *Fagopyrum*. Because the molecular clock assumption for the land plant lineages was rejected by the ML method (the KH test), I used the Bayesian approach that explicitly models molecular evolution. This method has advantages of putting several constraints on node times at once and of estimating confidence interval. Additionally, I used the ML method with assuming local molecular clocks for date estimation in *Fagopyrum*. In this thesis, I discussed *Fagopyrum* evolution particularly for the urophyllum group, highlighting the occurrence of hybridization and allopolyploidization and the time scale of evolution potentially related to the uplift of the Tibetan plateau on the basis of the multiple gene analysis.

(審査結果要旨)

本学位論文では、ソバ属 (*Fagopyrum*, Polygonaceae) 内の系統関係と分岐年代の推定について述べられている。ソバ属の系統関係は、これまでに葉緑体遺伝子を用いた分子系統樹解析によってかなり解明されていたが、遺伝子系統樹ごとのトポロジーの違いについての統計的検定はなされていなかった。また進化の年代推定についても、指標となる近縁植物の化石が未知であることから、研究は進んでいなかった。本論文では、遺伝様式が葉緑体 DNA とは異なる核 DNA を解析に取り入れ、ソバ属 *urophyllum* グループに属する 13 種の間系統関係が最尤法によって推定された。各遺伝子系統樹のトポロジーの違いについての統計的検定が行われ、種間雑種として *Fagopyrum rubifolium* が生じた可能性が示唆された。また、陸上植物全体の進化の時間的枠組のなかで、ソバ属進化の年代推定が行われた。陸上植物ではいくつかの分岐について化石記録から比較的信頼性の高い年代が知られているので、そのような情報を取り入れ、さらに系統による分子進化速度の変動を考慮することができるよう、Thorne-Kishino のベイズ法による解析法を用い、異なる 3 つの遺伝子データから分岐年代が推定された。ソバ属の進化は、およそ 3000 万年前に始まり、種間交雑を経て多様性を増したという可能性を示唆する新たな知見が得られた。また推定された年代と地史的な環境との関連も考察されている。

これまで陸上植物進化の時間スケールに関しては、解析に用いる遺伝子や解析方法によって推定年代が食い違っており、非常に混乱していた。本論文では高度な解析方法を用いて 3 つの異なる遺伝子について互いに矛盾のない年代が得られている。従ってここで得られたソバ属進化の推定年代も信頼できるものと考えられる。本論文には多くの新たな遺伝子配列データが含まれている。それを先行研究によって得られていたデータと併せて先端的なデータ解析法を駆使して解析し、多くの興味ある結果を得ている。

以上の評価より、本論文の内容は博士 (学術) に十分に値するものであると判断した。なお、本学位申請論文の内容に関する 1 編の原著論文 (申請者が筆頭著者) が、国際学術誌である *Genes & Genetic Systems* にすでに掲載されている。