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学 位 論 文 題 目 Phylogenetic and population genetic analyses of Zosteraceae species based on *rbcL*, *matK* and *phyA* nucleotide sequences: Implications for the origin and diversification of seagrasses in Japanese waters

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博士論文の要旨

Seagrasses are composed of four families (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae) belonging to angiosperms, and are thought to have adapted independently to aquatic life. Seagrass beds are productive ecosystems; estimates of average net primary production place seagrass beds as one of the most important in the biosphere. Seagrass beds also support high biodiversity and productivity of coastal waters. Rapid losses of seagrass beds, due mainly to artificial disturbances, endanger these species and associated ecosystems.

The seagrass family of Zosteraceae consists of three genera: *Phyllospadix*, *Zostera* and *Heterozostera*. This family is distributed mainly in temperate regions of the northern and southern hemispheres. There is no record of a Zosteraceae species occurring in both hemispheres, although some species in certain subtropical and tropical seagrass families (Hydrocharitaceae and Cymodoceaceae) occur in both hemispheres. High species diversity of Zosteraceae in the Northern Hemisphere is observed around the Japan Archipelago, suggesting the family may have evolved from this region. Unfortunately, many Zosteraceae seagrass beds have disappeared from Japanese waters. For instance, it is reported that seagrass beds have declined by 4% during the thirteen-year period from 1978 to 1991.

Conservation of these seagrasses requires that knowledge concerning the history and genetic variation of seagrass within and between species or local populations. To examine the indispensability of seagrass beds around the Japan Archipelago, I investigate the history and genetic diversity of Zosteraceae species from the viewpoint of population genetics.

First, 2.8 kb nucleotide sequences of *rbcL* and *matK* genes of the chloroplast genome were examined to elucidate the origin and subsequent diversification of the Zosteraceae family of seagrasses. I determined the 24 *rbcL* and 30 *matK* sequences from five *Zostera* species (*Z. marina*, *Z. caespitosa*, *Z. caulescens*, *Z. japonica*, and *Z. asiatica*). Furthermore, to determine the time at which Zosteraceae diverged from closely related species, I used 207 *rbcL* and 115 *matK* sequences of the order Alismatales, to which the four seagrass families and other ten families belong. The *rbcL* sequence data cover all 14 families of Alismatales, while the *matK* sequence data cover seven families. Using the nucleotide sequences from family Araceae as outgroups, the number of synonymous nucleotide substitutions were examined. After confirming the validity of a molecular clock of chloroplast genes between Zosteraceae and rice, I estimated the divergence times of Zosteraceae species by applying the synonymous substitution rate of the rice *rbcL* gene to Zosteraceae, with the following results.

1) The estimated divergence time between Zosteraceae and its closest relative, Potamogetonaceae, is approximately 100 - 133 million years (myr), placing it within the Cretaceous period. The divergence time between the genera *Zostera* and *Phyllospadix* was estimated as 36 - 48 myr. These values suggest that present Zosteraceae emerged somewhere in the period between 36 - 48 myr and 100 - 133 myr ago. 2) Two subgenera of *Zostera* - *Zostera* and *Zosterella* - exhibit monophyly and appear to have differentiated from each other

approximately 33 - 44 myr ago. However, the data indicate the third genus *Heterozostera* branched off only 5 - 7 myr ago from the stem lineage leading to *Zosterella*, which appears too recent in comparison with the ancient divergence of the two subgenera. 3) I estimated the emergence time of the most recent common ancestor of the subgenus *Zostera* as 6 - 8 myr ago. Based on these phylogenetic analyses, I propose a provisional age-related classification of the Zosteraceae to account for their origin and evolution. 4) I also examined the extent of polymorphisms in *Z. marina* using *rbcL* and *matK* sequences. Four haplotypes were found in the samples, which have undergone diversification during the past 1.5 myr. One haplotype is observed among samples taken from both sides of the Japan Archipelago, and closely related haplotypes are observed among samples taken from the eastern Pacific Ocean. Although the number of samples is not large, this result suggests that a simple genetic structure cannot explain the distribution of chloroplast haplotypes in seagrasses of Japanese waters and the Pacific Ocean.

Second, two seagrass species endemic to Japan and Korea, cosmopolitan *Zostera marina* and *Z. caulescens*, were chosen as a basis for population genetic analyses and from the two species I collected 29 and 10 samples, respectively. Sampling localities cover 16 and 6 local populations on both sides of the Japanese Archipelago for *Z. marina* and *Z. caulescens*, respectively. I sequenced the 1.5kb *phyA* gene in the nuclear genome and confirmed the suitability of the *phyA* gene as a molecular demographic marker with the following observations: 1) there are many segregating sites, 2) the region evolves in a neutral fashion, and 3) no recombination events were detected in the region. I then examined the genetic diversity of 58 *Z. marina* and 20 *Z. caulescens* *phyA* genes, focusing on population structure and the extent of gene flow among local populations.

Haplotypes found in *Z. marina* and *Z. caulescens* numbered 16 and 6, respectively. Both species possess unique characteristics. Phylogenetic tree analysis reveals that the 16 haplotypes of cosmopolitan *Z. marina* are classified into two groups, A and B, which diverged 1.4 myr ago. On the other hand, most haplotypes in endemic *Z. caulescens* are specific to each local population. However, the extent of local differentiation in *Z. caulescens* is not as great as that between the A and B haplotype groups of *Z. marina*. It also appears that each population within the species has not been panmictic during the time period in which the polymorphism appears. I also found a geographical association of haplotypes, suggesting limited amounts of gene flow between local populations. However, there are a few haplotypes shared between local populations, and even between populations in the Sea of Japan and the Pacific Ocean. These observations suggest that the observed population structure of the two species could not be explained solely by gene flow due to sea currents. This analysis also suggests that local disturbance, such as extinction of local populations, is likely to destroy the total genetic diversity of seagrasses in Japan.

The seagrass flora of Japan is characterized by the occurrence of several species of Zosteraceae that are considered endemic to the northwestern Pacific. Moreover, this study revealed that population structures are present in both populations of cosmopolitan *Z. marina* and the endemic species, *Z. caulescens*. The mechanism generating the genetic diversity of seagrasses

around Japan is not explained by a simple unidirectional gene flow by sea currents or geographical isolation, and a destruction of this habitat may take another 1 myr to restore the present genetic diversity.

論文審査結果の要旨

本学位論文は、4章から構成されている。まず第1章で、申請者の研究の目的が日本周辺域アマモの遺伝的多様性の解明であることを示している。第2章では、海草の起源について葉緑体遺伝子 (*rbcl* と *matk*) を用い、日本周辺域の海草 (アマモ) の分岐年代の推定を行った。この推定に稲葉緑体の塩基置換速度が適用できるかどうかを検定するため、シロイヌナズナを外群とした相対速度テストを行った。外群との間で飽和に達していない非同義置換を用い、年代推定を行うと、現存するアマモにいたる系統が最も近縁な科と分岐したのは白亜紀にさかのぼり、また属の分岐は 3000~4000 万年前、日本周辺域の各種アマモの分岐はおよそ 600 万年前と推定された。この分岐年代と日本海の成立過程の関連を議論した。第3章では、日本周辺域を含め、北半球に半損するアマモと日本周辺域固有種のタチアマモの集団遺伝学的解析を行った。日本周辺域とアメリカ西海岸の2地域を含めた16地点から29個体、タチアマモ10個体を採集し、それぞれ58染色体、20染色体について *phyA* 遺伝子の塩基配列を決定し解析した。その結果、アマモの *phyA* ハプロタイプは16種類、タチアマモのハプロタイプは6種類見つかった。アマモの16種類のハプロタイプはAグループ、Bグループに大別された。これらのハプロタイプの分布と最終地点の地理的な分布との間には相関があり、日本周辺域のアマモには集団構造があることを示した。また、これらのハプロタイプの期限が古いこと (140 万年前)、異なるハプロタイプが共存する集団があること、見つかったハプロタイプの頻度はAタイプが高いが、多様性の程度はBタイプのほうが高いことを考慮し、日本周辺域のアマモの起源と分布に関する仮説として、日本沿岸のアマモの一部が日本よりも南部の沿岸域 (おそらく中国南部沿岸域) から移入した可能性を示唆した。またアマモとタチアマモの地域特異性が高いが、遺伝的多様度という点ではアマモのほうが3倍程度大きいことを示し、この違いが2種の生態学的特性に基づく可能性を示唆した。第4章では、第3章で取り上げた日本周辺域のアマモの起源と分布に関する仮説を考察する上で重要な要因となる日本周辺域の海流の分布、及びその他の海棲の生物集団での遺伝的分化の研究について議論した。最後に、今後の展開として、さらに広範囲でのアマモの遺伝的多様性を明らかにするとともに、提示した仮説を検証するために日本周辺域での遺伝的多様性の特性をさらに詳細に解析する必要性を示した。

ここで得られた多くの知見は新しいものであり、今後のこれをもとにした研究の更なる発展が期待できる。以上の評価より、本論文の内容は博士 (理学) に十分値するものであると判定した。なお、本学位申請論文の内容に関する1篇の原著論文 (申請者は筆頭著者) が、国際学術誌である *Genes and Genetic Systems* にすでに掲載されている。また、公開の論文発表会では、研究内容がわかりやすくまとめられ、質疑応答も適切なものであった。審査委員による口頭試問においても集団遺伝学、分子進化学等生命体科学及び関連分野に関して十分な能力を持っていると認められた。また、論文の英語が明快であることから判断して十分な英語力を有することも認められた。以上の点から申請者は試験に合格したと判断した。