

題目 : Ecological speciation and genetic traits of the genus *Pittosporum* in the Ogasawara Islands (小笠原諸島のトベラ属における生態的種分と遺伝的特性)

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This research investigated the evolutionary history and conservation challenges of four endemic *Pittosporum* species inhabiting the Ogasawara Islands, a unique oceanic archipelago renowned for its exceptionally high levels of biodiversity and endemism. The study employed a multi-faceted approach, integrating phylogenetic analyses, population genetic studies, detailed ecological observations, and conservation considerations to elucidate the mechanisms driving speciation and to formulate effective conservation strategies for these vulnerable plants. The findings offer valuable insights into the evolutionary dynamics of oceanic island species and underscore the critical need for comprehensive conservation approaches tailored to the unique challenges posed by these fragile ecosystems.

Chapter 1: Molecular Phylogeny of Insular Endemics of *Pittosporum* (Pittosporaceae) on the Ogasawara Islands

The initial chapter established the phylogenetic relationships of the four endemic *Pittosporum* species within the broader evolutionary context of the genus. A comprehensive phylogenetic analysis was undertaken, utilizing the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and a substantial dataset encompassing numerous *Pittosporum* species from across their global distribution. This analysis yielded a pivotal finding: the four Ogasawara endemics formed a well-supported monophyletic group, strongly suggesting that their speciation occurred *in situ*, within the Ogasawara Islands themselves. This conclusion effectively rejects the alternative hypothesis of long-

distance dispersal followed by independent speciation events on the islands. The monophyletic nature of the Ogasawara endemics provides compelling evidence of a shared ancestor and subsequent diversification on the islands.

While this analysis successfully established the monophyletic nature of the Ogasawara *Pittosporum*, the limited resolving power of the ITS marker hindered a precise resolution of the interspecies relationships. The phylogenetic tree did, however, successfully identify the closest relatives to the Ogasawara endemics as species found in mainland Japan, China, and Taiwan, thereby providing valuable biogeographic context and highlighting the potential dispersal routes. These findings form a crucial foundation for the subsequent chapters, guiding the selection of appropriate analytical methods and setting the stage for more detailed investigations into the genetic relationships and ecological factors shaping speciation in the Ogasawara Islands.

Chapter 2: Population structure and dynamics of *Pittosporum* in the Ogasawara Islands using genome-wide SNPs

Recognizing the limitations of the ITS-based phylogenetic approach in resolving fine-scale relationships, Chapter 2 employed a far more powerful genomic method, ddRAD-seq, to conduct a significantly more detailed analysis of interspecific relationships and historical population dynamics. This approach offered substantially improved resolution compared to ITS-based analyses, providing crucial insight into gene flow patterns and the timing of speciation events.

The ddRAD-seq analysis decisively confirmed the monophyletic nature of the four endemic species and produced a highly resolved phylogenetic tree. This tree provided a significantly enhanced view of the evolutionary relationships, resolving the

precise branching order among the four species. Importantly, it established that *P. boninense* and *P. chichijimense* form a sister-group pair, as do *P. beecheyi* and *P. parvifolium*. This phylogenetic framework informed the subsequent analyses of population structure and gene flow.

A particular focus was placed on understanding the evolutionary history of the parapatric sister species, *P. boninense* and *P. chichijimense*, which share a relatively narrow zone of sympatry within the Chichijima Island's eastern forest region. The analysis revealed significant evidence of gene flow between these two species, implying that their divergence was not entirely driven by complete geographic isolation. Rather, the speciation process appears to involve a complex interplay of both genetic and ecological factors. The results demonstrate that ongoing and historical gene flow has significantly influenced their evolutionary trajectories, highlighting the intricacies of speciation in small, isolated island ecosystems where the influence of geographical distance is diminished.

Chapter 3: Ecological speciation factors of two endemic sister species of the genus *Pittosporum*, parapatrically distributed on Chichijima Island.

Chapter 3 directly addresses the role of ecological factors in driving speciation, using a detailed comparative analysis of the two closely related, parapatric sister species, *P. boninense* and *P. chichijimense*. The aim was to identify the key ecological traits that contribute to their reproductive isolation and species integrity. A broad range of phenotypic traits was measured across numerous individuals of both species, including leaf morphology (chlorophyll a/b ratio, leaf area, leaf mass per area, palisade thickness), photosynthetic performance (light response curves, dark respiration rate, light

compensation point, light use efficiency, maximum stomatal conductance), and environmental conditions (canopy openness, soil moisture, soil temperature).

The comparative analysis revealed significant phenotypic differences between the two species, reflecting adaptations to distinct microhabitats. *P. boninense*, frequently found in open or edge forest habitats, exhibited traits indicative of sun-adapted species (e.g., higher light use efficiency, and maximum stomatal conductance). In contrast, *P. chichijimense*, primarily found in shaded understory environments, displayed characteristics consistent with shade tolerance (e.g., larger leaf area). The observed differences in photosynthetic characteristics were consistent with the habitats each species occupies and strongly suggest adaptation to differing light environments has played a crucial role in their speciation.

Furthermore, the analysis of flowering phenology revealed a significant temporal mismatch in flowering times between the two species, a key mechanism of prezygotic reproductive isolation. *P. chichijimense* flowers earlier than *P. boninense*, limiting opportunities for interspecific hybridization and thereby enhancing their reproductive isolation. The reciprocal light exposure experiment further highlighted the differing tolerances of these species to high-light conditions, providing strong evidence for ecological adaptation as a driving force in their speciation.

Chapter 4: Potential of ex-situ conservation strains revealed by genetic analysis of Oceanic Islands' endangered species *Pittosporum parvifolium*

This chapter focuses on the urgent need for effective conservation strategies for *P. parvifolium*, a critically endangered species represented by only four extant individuals in its natural habitat. The study aimed to assess the genetic diversity of both wild and ex-

situ populations, provide insights into their genetic integrity, and suggest practical conservation measures to support their long-term survival.

Using a combination of SSR markers and population genetic analyses, this chapter explored the genetic diversity of *P. parvifolium*. Despite its drastically small population size, the wild *P. parvifolium* individuals exhibited remarkably high levels of genetic diversity and heterozygosity, potentially indicating that recent population bottlenecks have had a limited effect on its genetic variation. Furthermore, ex-situ populations cultivated in a botanical garden possessed unique alleles not found in the wild, emphasizing the importance of both in situ and ex-situ conservation approaches.

In situ conservation efforts included propagating seedlings from wild seeds over several growing seasons. These efforts revealed higher genetic diversity in seedlings produced through outcrossing than selfing, underscoring the importance of promoting outcrossing in the wild. This information is critical for informing future conservation strategies. The chapter recommends a comprehensive conservation approach that combines in-situ and ex-situ efforts. This strategy should include active management to maintain high genetic diversity, potentially involving carefully controlled outcrossing events to introduce unique alleles from the ex-situ population into the in-situ population. This multi-pronged approach aims to maximize genetic diversity, bolster population size, and enhance the long-term survival prospects of this critically endangered species.

In summary, this research provides a detailed and comprehensive understanding of the speciation mechanisms and conservation challenges associated with four endemic *Pittosporum* species on the Ogasawara Islands. The study demonstrates the significance of integrating phylogenetic and population genetic analyses with detailed ecological and

conservation considerations. The findings offer invaluable insights into the evolutionary biology of oceanic island species, highlighting the complex interplay between genetic and ecological factors shaping speciation and emphasizing the critical need for comprehensive, well-informed conservation strategies tailored to the specific challenges presented by these unique island ecosystems. This work offers a model for future research integrating evolutionary biology, ecology, and conservation to guide effective management of critically endangered species.