

# Evolutionary history and conservation genetics of *Primula* sect. *Reinii* (Primulaceae), an endangered endemic primrose group in Japan

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## Summary

The Japanese Archipelago has one of the highest levels of plant diversity in temperate forests in East Asia, and is characterized by high endemism. Evidence from the fossil record and molecular studies have suggested that high plant diversity in east Asia resulted from evolutionary diversification in relation to insularity produced by Quaternary sea-level changes and a historically moist climate that lacked glaciation. On the other hand, recent studies indicate that the highly endemic flora on the Japanese Archipelago was formed due to geohistorical factors, such as topographic and geographic heterogeneity. In fact, many molecular phylogeographic studies have elucidated that past geoclimatic events in the archipelago sculptured intraspecific genetic differentiation and contributed to the establishment of high plant diversity and endemism. Nevertheless, the extent to which geographic and climatic factors have driven speciation among closely related taxa in insular habitats is not adequately understood because most studies have focused on single species. Therefore, to identify the processes responsible for the biodiversity on the islands, the speciation history of a single plant group endemic to Japan should be investigated.

In this thesis, I have focused on *Primula* sect. *Reinii* (Primulaceae), which comprises four species endemic to Japan. These perennial herbs occur on all four main islands (Hokkaido, Honshu, Shikoku, and Kyushu), mainly on wet shaded rocky cliffs on mountains. Due to the low migration and dispersal abilities characteristic of edaphic endemic plants, *Reinii* species are thought to have been strongly affected by historical geoclimatic events in the Japanese Archipelago. Hence, this plant group is ideal for assessing the relative roles of topographic and climatic factors in speciation processes. Meanwhile, the low dispersal abilities of these plants may be disadvantageous when local conditions change. In fact, based on the rarity and narrow distribution range of each taxon, all taxa in sect. *Reinii* are listed as “threatened species” on the latest Japanese Red List. Because the diversity of plants in Japan is supported by the richness of geographically narrow endemics, this plant group is also useful for conservation biology of endangered Japanese plant groups. In the work presented in this thesis, I aimed to (1) elucidate the evolution and diversification of the sect. *Reinii*, and (2) obtain useful insight to develop an effective conservation plan for the endangered endemic primrose group.

In Chapter 1, I reconstructed the molecular phylogenies of the genus *Primula* and within sect. *Reinii* using multiple chloroplast and nuclear DNA regions to clarify the phylogenetic positions and relationships of section *Reinii*, interpret the biogeographic and diversification patterns of this group, and gain a better understanding of the role of climatic niche evolution of the section. The monophyly of sect. *Reinii* was supported by both chloroplast and nuclear trees. Fossil-calibrated dating analysis estimated that this section diverged from its sister taxon, sect. *Cortusoides*, around 1.82 million years ago (Ma). In addition, its lineage diversifications were accelerated after 1.0 Ma. This timeframe was characterised by Quaternary climatic oscillations and uplift of high mountains in Japan. Biogeographic inference suggested that a migration scenario in which sect. *Reinii* originated in Hokkaido and migrated to southerly regions of the Japanese Archipelago during the mid-late Pleistocene. These results indicate that spatiotemporal heterogeneity in the Japanese islands may play a significant role in the biogeographic history of Japanese endemic primroses. Furthermore, models of climatic niche evolution indicated that these closely related species, *P. tosaensis* and *P. reinii*, have contrasting niche optima and rates of niche evolution, with faster climatic niche evolution in *P. tosaensis* than in *P. reinii*. This implies that climatic niche divergence between the sister taxa played a key role in their speciation. Contrasting evolutionary processes found in closely related species illustrate the effects of climatic niche evolution on

species' diversification patterns under severe climatic and topographic changes during the Quaternary.

In Chapter 2, I applied population genetic analyses and species distribution modeling to investigate the evolutionary history of two ultramafic *Primula*, *P. hidakana* and *P. takedana*, which are ecologically distinct sister taxa with disjunct distributions on Hokkaido Island, Japan. *P. hidakana* is found on various rocks in southern Hokkaido and *P. takedana* in serpentine areas in northern Hokkaido. The results of Bayesian clustering analysis using multiple nuclear DNA microsatellite loci demonstrated significant genetic divergence between *P. takedana* and *P. hidakana*, and the existence of two distinct genetic units corresponding to each species. On the other hand, no significant haplotype divergence between the species was found in chloroplast DNA. Approximate Bayesian computation (ABC) based on a nuclear data set supported the secondary contact scenario (gene flow subsequent to an initial allopatric phase of divergence), and indicated that *P. takedana* evolved from *P. hidakana* approximately 112,000 years ago, corresponding to the last interglacial (LIG) period. These results indicate that the serpentine endemic *P. takedana* originated via a path way of biotype depletion, suggesting that the serpentine areas on northern Hokkaido served as refugia for their ancestral populations during the LIG period. Asymmetrical gene flow from *P. hidakana* into *P. takedana* is attributable to the secondary contact during the last glacial (LG) period. Paleodistribution modeling based on climatic and geological data supported a model of divergence in allopatric and latitudinal range shifts. Thus, this study suggests that the late Pleistocene climate and island-like chains of serpentine areas on Hokkaido Island may have played a major role in the relatively rapid divergence process of the two sister primroses.

In Chapter 3, I compared the floral morphologies and pollinator fauna of two sister taxa, *Primula hidakana* and *P. takedana*, to obtain a better understanding of the pollination systems of these species. Observations of their floral characteristics revealed that both species are strictly distylous. The flower shape of *P. hidakana* has a much larger corolla than that of its sister species, and the diameter of its corolla tube mouth was approximately half that of *P. takedana*. Observations of flower visitors revealed that pollinators of *P. takedana*, which were small, short-tongued insects, varied more than those of *P. hidakana* did. The diversity of *P. takedana* pollinator fauna implies that the primrose is unable to restrict the type of insect visitor it receives due to flower structure constraints. This study suggests that differences in floral structure between these sister primrose species are associated with pollinator fauna differentiation, which may have contributed to their speciation.

In Chapter 4, to develop an effective conservation plan for *Reinii* species, I investigated the genetic status of ex situ *P. reinii* var. *rhodotricha* and related species using multiple nuclear microsatellite markers. As expected, the level of genetic diversity indicated by allelic richness and heterozygosity was lower for *P. reinii* var. *rhodotricha* than for wild populations of related species. This provides molecular evidence for the recent critical status designation of the ex situ population. However, despite their low genetic diversity and small population size, no inbreeding was detected in ex situ populations of *P. reinii* var. *rhodotricha*. Furthermore, the inbreeding coefficients and population size were positively correlated among wild *Reinii* species, indicating that higher fitness is associated with heterozygosity. These results imply that genetic purge may lead to a genetic decline in smaller populations of these species, which likely suffer strong inbreeding depression, similar to several other *Primula* species. Thus, this study highlights that importance of genetic assessment for conservation of endangered endemic plant species with small, long isolated populations.

Based on findings from these studies, it is suggested that the high geological and environmental (abiotic) heterogeneity in the Japanese Archipelago affected the mode and tempo of diversification of *Primula* sect. *Reinii*. The evolutionary history of the section demonstrates that the plant diversity and endemism in the Japanese Archipelago have expanded as a result of allopatric speciation processes, which led to region-specific abiotic conditions. In addition, this insight into evolutionary diversification of plants has important implications for conservation of biodiversity hotspots. This study emphasizes the importance of approaching conservation biology from an evolutionary perspective.