

Evolution and diversification of the section *Brachycalyx* (*Rhododendron*, Ericaceae) inferred by phylogenetic and population genetic approaches

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Summary

The distributions of many plant species changed as a result of the climatic oscillations during the Quaternary, and because of this vicariance may have occurred within some species. As a consequence, allopatric speciation and diversification may have been promoted even in woody species with slow rates of evolution. Continental islands can provide suitable settings in which to examine the effects of geographical isolation on evolution and diversification via allopatric processes that took place in plant species during the Quaternary. The section *Brachycalyx* Sweet (*Rhododendron*, Ericaceae) contains broad-leaved tree and shrub species, which are distributed in continental islands (the Japanese Archipelago, Jeju Island, and Taiwan) and in mainland China, and it has greatly diversified in the Japanese Archipelago. The section is therefore an attractive taxon for exploring evolutionary processes in woody species on continental islands. In the work presented in this thesis, I aimed to elucidate the evolution and diversification of the section *Brachycalyx* from a phylogeographic perspective, using phylogenetic and population genetic approaches.

In the research described in Chapter III, I studied phylogeographic history within the study taxon by using chloroplast DNA sequences to examine phylogenetic relationships among species and haplotypes, their geographical distribution, and the level of genetic diversity in each species and the extent to which it differed between continental islands and the mainland. The most recent common ancestor of sect. *Brachycalyx* probably started to diversify from the late Pliocene onwards. Although the distributions of continental species and insular species did not overlap, monophyly within each region was rejected on the basis of the results. Many haplotypes were recognized in the species on the Japanese Archipelago, while in many cases the insular species showed no variation within a population. Through analysis of molecular variance,

the percentage of variation among populations within the insular species was found to be higher than the percentage of variation within populations, while the percentage of variation among populations within the continental species was lower than that of variation within populations. This suggests that populations of the insular species may have been influenced by genetic drift. The diversification of this section in the Japanese Archipelago is characteristic of the high level of species diversity in East Asia, which may have been caused by allopatric speciation due to geographic isolation during the Quaternary.

In Chapter IV, I present the results of studies on demographic history of *R. weyrichii*, a species which is widely distributed across different continental islands, using statistical phylogeographic methods based on chloroplast and nuclear DNA sequences and ecological niche modeling. Clear genetic divergence between Shikoku and Kyushu Islands was observed according to both nuclear and chloroplast DNA sequence analyses, and the population divergence time was estimated to date back to before the last glacial period. The estimates of migration rates between the islands after divergence were low, suggesting that the sea floor that was exposed during the last glacial period may not have provided effective corridors for dispersal, and thus regional population divergence was promoted. This idea was also supported by the results of palaeodistribution modelling, which showed that the range of the species was fragmented among the islands even during the LGM. These findings may help us to understand population history and the effects of land bridges on the biogeography of continental islands.

In Chapter V, I examined the differences in genetic diversity and population genetic structure between a common species (*R. weyrichii*) and two rare species (*R. sanctum* and *R. amagianum*), using population genetic analyses based on genotyping at nuclear microsatellite loci. As expected, the level of genetic diversity indicated by allelic richness and gene diversity was lower for the rare species *R. sanctum* than for the common species *R. weyrichii*. Analyses of the isolation-by-distance pattern, neighbor-joining trees, and Bayesian clustering indicated that *R. sanctum* had a strong population genetic structure whereas *R. amagianum* exhibited very weak genetic structure among populations, and that there was moderate population genetic structure in *R. weyrichii*. The degree and pattern of population genetic structure in each species was therefore unrelated to its rarity and instead merely reflected its geographic distribution.

Based on findings from these three studies, it is suggested that genetic divergence due to allopatric processes and vicariance was responsible for the diversification of sect. *Brachycalyx*. The rapid diversification of this section from the late Pliocene onwards and the current species diversity in the Japanese Archipelago are striking, even amongst the highly

diverse woody species of East Asia. It is likely that populations of many species in the section were affected by processes such as isolation between islands as observed for *R. weyrichii* and vicariance within islands as was found in the case of *R. sanctum*. Such an allopatric history including isolation or vicariance may have created and maintained species diversity and genetic diversity and structure within species in this section during the Quaternary. Information on genetic diversity and population genetic structure within individual species will be a useful tool in conservation efforts directed towards rare species in the section.