

Genetic and ecophysiological consequences of habitat diversification
in *Saxifraga fortunei* (Saxifragaceae)

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Summary

How the phenotypic diversity of organisms has been generated in heterogeneous natural landscapes is a fundamental question in evolutionary biology. Many plant species exhibit various phenotypes depending on the species and population in response to the surrounding environment. The evolution of these diverse phenotypes is mainly a result of adaptation to novel habitat environments. By altering the phenotype, the structural, physiological, and ecological performances can be optimized to the habitat conditions, resulting in higher fitness and flourishing of the species. Such adaptive diversification could be driven by divergent natural selection and neutral genetic processes in past population demography. When similar phenotypes develop in independent regions under the same environments multiple times, it provides strong evidence for adaptation as parallel evolution. In contrast, a random process of genetic drift in past biogeographic history could also contribute to phenotypic diversification. In a small population, founder and bottleneck effects could promote drastic phenotypic evolution. In this case, phylogenetic and population genetic analyses are effective methodologies to reveal the evolutionary pattern of phenotypic diversity. However, it is also important to identify the functional mechanisms generating phenotypic differences in a specific environment. Individuals interact with the surrounding environment via physiological and ecological relationships using genetically regulated phenotypes. During environmental adaptation, one phenotype with higher fitness becomes dominant in a population, leading to population divergence with functionally differentiated phenotypes. Therefore, investigating both phylogenetic patterns and functional mechanisms would lead to a comprehensive understanding of phenotypic diversification as an evolutionary response.

Section *Irregulares* Haw in *Saxifraga* L. is a well-characterized group with zygomorphic flowers and is the earliest diverged lineage. This section consists of more

than 18 species growing in moist temperate areas of East Asia, and most of them show limited distribution in local areas. *Saxifraga fortunei* Hook. f. complex has the widest distribution area in this group and is distributed in East Asia, including mainland China, the Korean Peninsula, the Japanese Archipelago (from Hokkaido to Yakushima Island), Far East Russia, and Sakhalin. Interestingly, plants in the Eurasian region grow stably in moist and shaded understory with specific morphologies, whereas *the S. fortunei* complex in the Japanese Archipelago harbors diverse habitats and ecological characteristics. They grow in various environments, such as riverside, alpine zone, and remote islands, in addition to the forest floor, showing specific morphologies, particularly leaf shapes, and ecological functions corresponding to their habitats. The various ecotypic entities were expected to have evolved from the ancestral population due to local adaptation after colonization in Japan. In particular, because riverside and alpine ecotypic taxa showed fragmented distributions in the northern part of Japan, they may have arisen in parallel in independent regions. Therefore, the *S. fortunei* complex is a suitable model for investigating the phenotypic diversification corresponding to the adaptation to various habitat environments. In this thesis, I aim to elucidate the evolutionary patterns and mechanisms of phenotypic diversity corresponding to various habitats in the *S. fortunei* complex and its related species.

In Chapter 1, I investigated the biogeographic history of the *S. fortunei* complex to address the phenotypic diversification process in past population demography. I also determined the phylogenetic origins and whether ecotypic taxa have single or multiple origins. Sanger sequencing-based analysis using nuclear and chloroplast DNA was used to estimate the divergence timeframe within the complex, and genome-wide single nucleotide polymorphisms (SNPs) obtained by the double digest restriction site associated DNA sequencing (ddRAD-seq) were used to investigate the phylogenetic origins of ecotypic taxa. Phylogenetic analyses revealed the divergence of the Japanese population from the continental population in the late Miocene. Two distinct regional clades of North and South Japan were identified; phenotypic diversification was evident only in the southern clade. The South Japan clades displayed a historical distribution expansion from north to south. Phenotypic variations appeared to have been generated during expansion. The ecotypic boundaries were incongruent with the genetic grouping.

I propose that morphological and ecological specialization in Japanese populations was repeatedly generated by local natural selection.

Phenotypic divergence often reduces resource competition in the sympatric population, leading to an increase in and maintenance of species diversity within an ecosystem. In Chapter 2, I assessed the impact of phenotypic and habitat differences on genetic and ecological consequences using *S. fortunei* and its sister taxon, *Saxifraga acerifolia* Wakab. et Satomi. Although ecologically similar, they showed sympatric distribution with habitat segregation within the two river systems in Japan. They grow in different microhabitats with leaf morphological variation: *S. fortunei* with broadly ovate and deep green leaves is distributed continuously along the riverside, whereas *S. acerifolia* with deeply dissected and bright-colored leaves grows on the rocky surface of waterfall. Therefore, the differentiation of genetic structures is influenced by their spatial population arrangements based on habitat preferences. I compared their genetic structures using chloroplast microsatellite variations and genome-wide SNPs obtained by multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq). I also estimated gene flow among and within populations and performed landscape genetic analyses to evaluate seed and pollen movement and the extent of genetic isolation related to geographic distance and habitat differences. I found a strong genetic structure in *S. acerifolia*, even on a small spatial scale (< 1 km part); each population on each waterfall in the same river system had a completely different predominant haplotype. In contrast, *S. fortunei* showed no clear genetic differentiation. My findings suggest that the level of genetic isolation was increased by spatially fragmented habitats and due to limited seed and pollen dispersal of *S. acerifolia* over waterfalls. Habitat differentiation between the sister taxa could have contributed to the different patterns of gene flow and shaped the contrasting genetic structures.

In Chapter 3, I investigated the adaptive mechanisms that generate phenotypic differences between populations. Among several environmental factors characterizing various habitats, I focused on light conditions because this environmental factor varies significantly among populations and has a significant impact on plants' survival. The sun ecotype growing in grassland (exposed habitat) and shade ecotype growing in a forest floor (shaded habitat) were used for the cultivation experiments and anatomical and ecophysiological analyses. Leaf anatomical and physiological traits were measured in

natural habitats and two common gardens (high- and low-intensity light experimental sites) to detect the genetic effects of phenotypic differences. Comparing the level of photoinhibition of PSII and leaf mortality rate under high-light conditions, I assessed the ecological impacts of natural selection. In addition, population genetic analysis was conducted to investigate the genetic differentiation between ecotypes. I found different photosynthetic traits, such as different leaf anatomical structure and ecophysiological performance in the sun and shade ecotypes, with a close geographic arrangement and a new phylogenetic origin. Specifically, well-developed palisade parenchyma increases photosynthetic rates under high-intensity light and reduces the level of photoinhibition by high-intensity light. Therefore, as strong selective pressure, high-intensity light stress would rapidly promote phenotypic divergence between ecotypes, particularly in traits deeply related to adaptation to different light conditions.

In summary, I investigated the evolutionary patterns and functional mechanisms that drive phenotypic diversification corresponding to various habitats in the *S. fortunei* complex and its related species. I found that adaptive processes under divergent natural selection, in addition to random genetic drift in past population demography, could be a driver of phenotypic diversification. In contrasting habitat environments, adaptive traits were repeatedly shifted, leading to physiologically and ecologically distinct populations. The phenotypic diversity could have contributed to the wide distribution range of the *S. fortunei* complex and its coexistence with congeneric species even in a sympatric distribution. These results highlight the high evolutionary potential of the *S. fortunei* complex, which corresponds to natural selection as a powerful trigger for phenotypic diversification.