

Summary

The climatic oscillations of the Quaternary, characterized by repeated glacial and interglacial periods, have had a significant impact on plant population dynamics. It is believed that many temperate plant species responded to these climatic cycles by forming refugia in the southern regions, which remained relatively warm during the glacial periods, and expanding their distribution northward during the interglacial periods. This pattern of range shifts is also applicable to the temperate forests of the Japanese archipelago. During glacial periods, southwestern regions served as stable refugia, while the northeastern regions experienced range contraction, with palynological studies suggesting that many of the populations currently inhabiting the northeast arose from northward postglacial expansion. Genetic analyses also support this hypothesis: long-term population stability in the southwestern regions has resulted in high genetic diversity in both chloroplast and nuclear genomes, whereas the northeastern regions exhibit lower genetic diversity due to population bottlenecks during the glacial periods and founder effects experienced during the post-glacial range expansion process. This pattern has been reported for multiple cool-temperate tree species in Japan. Thus, current genetic diversity is closely tied to the history of population dynamics, particularly reflecting the influence of climatic changes from the Last Glacial Period to the present. However, genetic diversity is not solely influenced by climate; it is also affected by various factors such as generation time, mating systems, and seed dispersal mechanisms. Additionally, the impact of climate change likely varies by species, meaning that plants growing in the same climatic zone may not have necessarily experienced similar population dynamics. Therefore, phylogeographical studies of diverse plants with different life histories are needed for a more comprehensive discussion of the history of plant distributional changes in response to climatic oscillations. Nevertheless, previous studies have been biased toward trees, and there have been few studies on herbs, whose life histories differ significantly from those of trees. In the case of Japan, vegetation is mainly controlled by temperature, and temperate herbs may have experienced similar distributional transitions to trees growing in the same climatic zone. On the other hand, because of their high sensitivity to climate change, herbs may have experienced unique population dynamics in response to climatic oscillations. Particularly, rear-edge populations located at the distribution limits of a species are crucial for studying the relationship between genetic diversity and climate change since the Last Glacial Period, as they are the first to experience and endure the long-term effects of climate shifts.

The aim of this study is to elucidate how temperate herbaceous plants in Japan have responded to glacial-interglacial cycles in terms of their distributions from a comparative phylogeographic perspective. Focusing particularly on rear-edge populations, I examine the impact of climate change on these populations through genetic methods and ecological niche modelling. Additionally, I

investigate similarities and differences in population dynamics among the three target species, as well as differences between herbaceous plants and trees.

In Chapter 1, the study focused on *Hemerocallis middendorffii* Trautv. et C.A.Mey (Asphodelaceae), a perennial herb with its core distribution in temperate grasslands. This chapter aimed to clarify the impact of climate change on the population dynamics of this species by focusing on rear-edge populations, analysing current genetic diversity, reconstructing demography, and investigating the correlation between historical distribution probabilities and genetic diversity. Genetic analysis using microsatellite markers was conducted on 737 individuals from 41 populations across Japan, revealing that the species is divided into six regional populations. Four regional populations, ranging from Hokkaido to the Chubu region, were widely distributed, exhibiting high genetic diversity and slight differentiation within the regions. In contrast, the two southwestern edge populations (Kyoto and Oki Islands) were geographically and genetically isolated from the other regions, showing reduced genetic diversity. Demographic analyses indicated a decline in effective population size across all regions, with the southwestern edge populations experiencing a significant reduction in size during the postglacial period. Furthermore, by statistically linking the independent results of ecological niche modelling and genetic analysis, it was shown that the estimated historical distribution during the Holocene Climatic Optimum (approximately 6,000 years ago) is a key factor influencing the current genetic diversity of the populations. These findings suggest that the postglacial warming and subsequent range contraction and fragmentation have significantly impacted the dynamics of the rear-edge populations of *H. middendorffii*, leading to reduced genetic diversity.

In Chapter 2, the focus is on *Arnica mallotopus* Makino (Asteraceae), a perennial herb that thrives in cool temperate regions. Given that its distribution closely corresponds to the heavy snowfall areas on the Sea of Japan side, it is believed to be sensitive to changes in snowy environments. Therefore, this chapter aims to elucidate the impact of past environmental changes on the distribution and population dynamics of *A. mallotopus* through genetic analysis and landscape analysis. Population genetic and landscape analyses were conducted using genome-wide SNPs and microsatellite markers across 28 populations covering its distribution area. The genetic analysis revealed that *A. mallotopus* is divided into four regional populations, with two in the north-south direction. All population differentiation occurred during the Last Glacial Maximum (LGM), and there was a reduction in effective population size after this differentiation. In eastern Japan, there was minimal differentiation between populations, and genetic diversity was relatively high, while in western Japan, significant differentiation among populations was observed, with a tendency toward lower diversity. Landscape analysis indicated a strong dependence on heavy snowfall for the distribution of this species. In eastern Japan, where heavy snowfall regions are contiguous, suitable habitats are widely distributed; conversely, in western Japan, suitable habitats are more limited. These results suggest that the current regional populations derive from different refugia formed during the LGM, with decreased temperatures and reduced snowfall likely serving as limiting factors for distribution. While the warming and increased snowfall in the postglacial period are believed to have facilitated the expansion of this species' distribution, the impacts

are inferred to have varied by region. Specifically, in eastern Japan, the distribution rapidly expanded and thrived alongside the enlargement of suitable habitats; however, in western Japan, the limited suitable habitats led to fragmentation of the distribution, causing the populations to be strongly influenced by genetic drift.

In Chapter 3, I focused on *Iris gracilipes* A. Gray, a perennial herb endemic to Japan. The distribution of this species closely aligns with that of cool temperate forests in Honshu. However, its distribution is fragmented, and populations are at risk of extinction in various locations. This chapter aims to clarify whether cool temperate herbs like *I. gracilipes* followed a distribution pattern similar to that of trees in response to climatic oscillations or whether they experienced specific population dynamics, using genetic analysis and ecological niche modelling. Genome-wide SNPs were obtained using MIG-seq from 75 individuals across 17 populations, revealing four geographically distinct regional populations. Furthermore, genetic diversity showed a significant correlation with latitude, with southern populations exhibiting lower diversity. Demographic analysis suggested that these regional populations had already been established by at least the LGM, with northern populations having a large and stable effective population size, while southern populations likely experienced a bottleneck during the postglacial period. Ecological niche modelling results indicated that suitable habitats for this species expanded steadily in the northern regions after the LGM, whereas in the southern regions, they shrank during the postglacial period, with further contraction anticipated due to warming. These results suggest that *I. gracilipes* maintained stable populations in high-latitude regions during the Last Glacial period, allowing it to preserve high genetic diversity. In contrast, in low-latitude regions, postglacial warming led to population contraction and fragmentation, which resulted in a significant impact from genetic drift and a decrease in genetic diversity. This geographic gradient of genetic diversity is the opposite of patterns reported for many cool temperate trees. Therefore, even within the same climatic zone, plants with different life histories, such as herbaceous plants and trees, exhibit different population dynamics in response to climate oscillations. Furthermore, the genetic structure and population-specific genetic diversity obtained from such phylogeographic analyses are expected to contribute to the conservation of this endangered species.

These phylogeographic studies revealed a common pattern regarding genetic diversity. Three cool-temperate perennial herbaceous species showed a pattern of genetic diversity higher in the north and lower in the south, the exact opposite of what has been observed in some cool-temperate tree species. These results suggest that low-latitude populations, such as the rear-edge population, are not stable throughout the glacial and postglacial periods as previously reported for temperate trees, but are unstable populations that experienced bottlenecks in the postglacial period for temperate grasses. Populations at high latitudes were more stable than those of temperate trees during the postglacial period, suggesting that these regional differences in population dynamics under climatic oscillations created a geographic gradient of genetic diversity that differed between herbaceous and tree species. Thus, my study shows that I cannot simply assume that all plants growing in the same climatic zone experienced the same pattern of population dynamics.