

## Speciation of the sect. *Camellia* based on Pollinator shift

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### INTRODUCTION

Organisms have the ability to adapt to specific conditions within their environments, resulting in morphological diversification and speciation. It is thought that speciation is more likely to occur as a result of pre-mating isolation rather than post-mating isolation. Therefore, to elucidate the speciation of plants, it is important to identify the factors that cause pre-mating isolation.

In Japan, *Camellia japonica* and *Camellia rusticana* belonging to the sect. *Camellia* are naturally distributed. Despite differences in their habitats and morphologies, they have been classified as variants, subspecies, or species depending on the researchers. Thus, their taxonomic positioning remains unclear, because they are known to hybridize if their habitats are adjacent to each other.

*C. rusticana* grows in snowy places, whereas *C. japonica* does not. While *C. japonica* adopts ornithophily, *C. rusticana* adopts entomophily. Both species have adapted to different growing environments and pollinators. Thus, they may have been speciated by pre-mating isolation, which causes morphological mutations in flowers and leaves. Hence, this study aims to examine the degree of speciation of two species by comparing the floral and leaf morphologies and the genetic differentiations.

In the results, it was possible to distinguish between these species by the presence or absence of hypodermis in leaf morphology. The population that had hypodermal tissue was predominantly found in the *C. rusticana*-distribution area, while the population without the hypodermal tissue was mainly found in the *C. japonica*-distribution area (Fig. 1).

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In flower morphology, PCA analysis clearly distinguished between these species, and both petal and filament colors were also statistically different between these species (Fig. 2). The cumulative contribution rate was about 87% on the PC 1 axis and about 96% on the PC 2 axis. On the PC1 axis, eigenvalues reached close to an upper -0.5, with the maximum length of the stamen, coalescence rate, and length of pistil.

The genetic structures based on eight cpSSR loci and 151 SNPs are divided into three: southern and northern *C.japonica*, and *C.rusticana*. The haplotype diversities are 3.13 in *C. japonica* and 30.8 in *C. rusticana*. These results suggest that morphological and genetic differences between these species are clear. Furthermore, we investigated the candidate pollinators quantitatively. They are birds for *C.japonica* and small insects for *C.rusticana*, which means the gene flow between two species might be rare.

In conclusion, it is reasonable to think that *C.japonica* and *C.rusticana* are different species and are not closely-related because the difference in morphology is not consecutive and there is no difference in the genetic diversity. It also suggested that pre-mating isolation caused by differences in environments is one of the factors that promote speciation of sect. *Camellia*. It could cause the pollinator shift between birds and insects. The main pollinators of *C.japonica* are birds because it blooms in winter when the availability of fruits and insects is limited in the temperate forests. On the other hand, *C.rusticana* are pollinated by small insects in the early spring season when insects begin to come out. Then the adaptation to different environments might result in the different pollination systems.

Fig. 1 A cross section morphology observation of leaves of *C.japonica* and *C.rusticana*

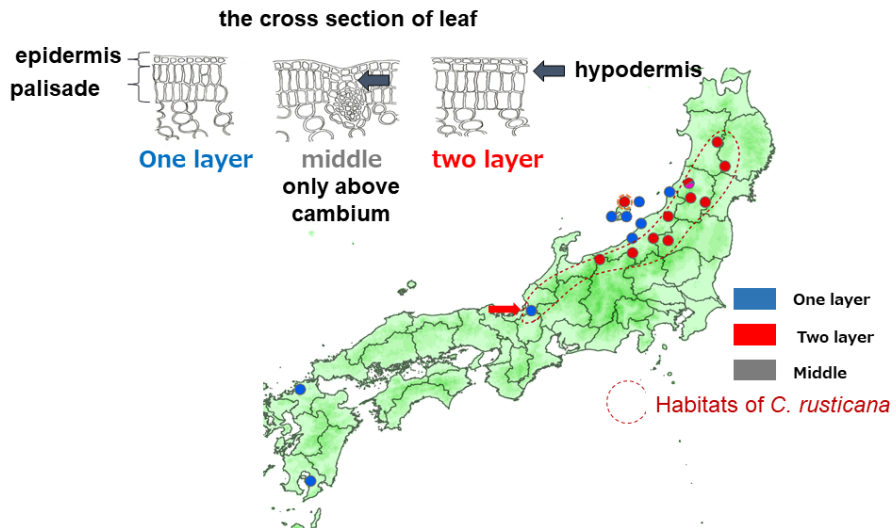
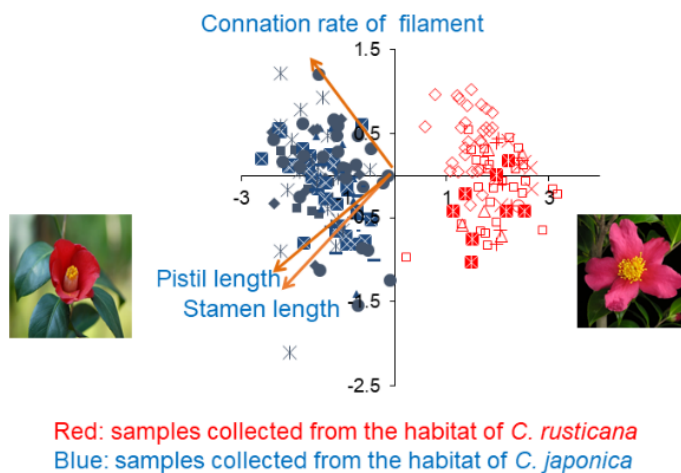


Fig. 2 PCA analysis using flower traits of *C. japonica* and *C. rusticana*



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