

## The genetic basis for *Lotus japonicus* cold adaptation and colonization of Japan

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During colonization of new regions, sessile plants face physical barriers limiting dispersal and environmental variation challenging phenotypic plasticity, but the genetics underpinning adaptation and colonization are poorly understood. Based on re-sequencing 136 wild *Lotus japonicus* accessions collected throughout Japan, we found that *Lotus* is a relatively recent addition to the flora of Japan. It likely migrated to southern Japan 50-60,000 years ago and reached northern Japan only a few thousand years ago, while differentiating into three distinct subpopulations and losing genetic diversity along the way. Colonizing northern Japan required adaptation to severe winter conditions, and using genome-wide association analysis we identified SNPs strongly associated with winter hardiness under field conditions. Remarkably, the same SNPs also clearly distinguished the central and northern subpopulations. This identifies winter hardiness as one of the traits that has driven population differentiation, and at the same time points to candidate genes and genomic regions associated with this specific trait. Our results suggest that phenotype-independent analysis of population differentiation coupled with in-field transplantation experiments represents a powerful approach for dissecting plant evolution and adaptation.