

論文要約

論文題目

Plant adaptive evolution at a limit of life: Phylogenetic, population genetic, and comparative genomic analyses of an extremophyte (*Carex angustisquama*, Cyperaceae) in highly acidic solfatara fields

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The study of adaptation has been playing a central role in evolutionary biology; however, detecting the mechanisms underlying adaptation remains tough even in the era of genomics. Extreme environments and plants thriving in them, called extremophytes, offer an ideal platform to overcome such a difficulty in adaptation researches because the limited number of extreme stressors often found in extreme environments can highlight the responsible selective agents and leave relatively clear signatures of selection on the genome, making it easier to identify both the phenotypic and molecular mechanisms underlying adaptation. Recently, increasing number of studies have exploited such relatively straightforward systems to uncover the diverse mechanisms underlying particularly high environmental adaptability in plants, some of which started to challenge the frontiers of evolutionary biology. However, these works have only been focusing on a few model plant species, and consequently, some extreme environments that prevent such model species from colonizing remain out of scope of evolutionary biological studies.

Here, I focused on the fumarole fields often found near the active volcanoes as such an overlooked extreme environment. In fumarole fields, ongoing volcanism leads some distinctive ecological parameters, such as atmospheric concentration of toxic pollutants, soil temperature, and soil acidity, to the extreme levels. In contrast to much attention from evolutionary microbiologists, the evolutionary aspects of higher plants in fumarole fields have been less explored. Thus, in this thesis, I especially focused on *Carex angustisquama* (Cyperaceae), one of a few dominant higher plants in solfatara fields where high concentrations of sulfur gases are continuously emitted. Because the sedge plant is found only in the closest area to fumaroles where the soil is heavily acidified (pH=2–3), *C. angustisquama* is an extremophyte in that it can survive at the end of plant habitable soil acidity. In addition, this species has no related species growing on similar soil conditions, offering a precious opportunity to investigate the evolutionary processes of the extremophyte via the adaptation to extreme soil acidity. Therefore, in this thesis, I aimed to comprehensively reveal the evolutionary processes of the extremophyte from macro to micro scales, and thereby gain some implications about the effects of environmental adaptation on plant diversification.

First of all, to grasp the macroevolutionary background of *C. angustisquama*, I reconstructed the phylogenetic relationships of sect. Phacocystis and Podogynae which harbor *C. angustisquama* and its related species using the genome-wide SNPs obtained from ddRAD-seq and MIG-seq (Chapter 1). The resulted phylogenetic tree showed that Japanese species from the two sections consisted of three subclades consistent with the

geographic ranges (i.e., Arctic, East-Asian continent, and Japanese endemic subclades). In addition, my macroevolutionary modellings suggested that historical processes of habitat shifts were associated with speciation processes within Japanese endemic subclades. Notably, colonization into wet slope created by heavy snow regime peculiar to Japan preceded habitat shifts within Japanese endemic subclades, suggesting the occupation of heavy-snow related habitat as a possible evolutionary precursor to generate the extremophyte in solfatara fields.

Next, I conducted population genetic analyses to reveal the detailed evolutionary processes of the extremophyte. Specifically, I examined the phylogenetic origins of the extremophyte, reconstructed past demographic history after speciation, and evaluated the direction and intensity of interspecific gene flow. These investigations clarified that *C. angustisquama* formed a fully-supported monophyletic clade with *C. doenitzii*, a species growing on non-highly acidic soils in subalpine areas in Japan. In addition, *C. angustisquama* was shown to have been suffered from the repeated founder effects associated with habitat specialization and subsequent population demography, resulting in drastic reduction of genetic diversity compared to the sister species (Chapter 2). To add insult to injury, interspecific gene flow has been restricted for a long time by habitat segregation (Chapter 3), preventing the supply of genetic variation from closely related species. As a result, the genome of *C. angustisquama* is strongly homogenized as a whole, providing an important example of genetic consequences of the adaptation to extreme environments.

The above results indicated that adaptation to solfatara fields played a critical role in the species formation and maintenance of the extremophyte. Thus, I next aimed to identify a responsible selective agent for the adaptation of *C. angustisquama* (Chapter 4). To achieve this, the combination of topographical surveys, soil analysis, and hydroponic culture experiments were taken place. These ecological approaches revealed a significant impact of extremely low pH, not Al^{3+} which is another major growth limiting factor in acidic soils, on determining the distribution limits of *C. angustisquama* within solfatara fields. This result was supported by the hydroponic culture experiments revealing the decreased root elongation below pH 2.0 which was accordant with the minimum pH values of the actual habitats of *C. angustisquama*. In addition, interspecific comparisons of tolerances to low pH and Al^{3+} highlighted that *C. angustisquama* exhibited higher tolerance to low pH stress than that of the sister species while there is no difference in Al^{3+} tolerance between the two species. These results indicate that novel adaptation to low pH stress played an important role in the adaptation of the extremophyte, while Al^{3+} tolerance was acquired prior to speciation of *C. angustisquama* and the sister species.

Based on the above results, I finally moved on to the genomic surveys for the low pH tolerance genes in *C. angustisquama* (Chapter 5). Firstly, I obtained Nanopore long reads from *C. angustisquama* (n=33 or 34) to newly construct the draft reference genome consisting of 41 contigs. Then, I performed comparative transcriptome analysis to reveal that several *STOPI* downstream genes, which have shown to play a critical role in *Arabidopsis* low pH tolerance, expressed in response to low pH stress both in *C. angustisquama* and its sister species. On the other hand, highly expressed genes only in *C. angustisquama* included class III peroxidase (CIII *Prx*) genes which are involved in antioxidation and cell wall organization. My comparative whole-genome analysis between *C. angustisquama* and *C. doenitzii* also showed that there was an insertion in *C.*

angustisquama (~500kb) where CIII *Prx* genes were duplicated. Based on these results, I finally tested the contribution of CIII *Prxs* activities to low pH tolerance in *C. angustisquama* with hydroponic culture experiments using CIII *Prxs* inhibitor. This pharmaceutical approach showed that the inhibitor caused the decrease in low pH tolerance of *C. angustisquama*. These results indicated that the duplication of CIII *Prx* genes played an important role in the adaptation of the extremophyte, and suggested CIII *Prx* genes as possible unknown low pH tolerance genes in plants.

Overall, my multiscale evolutionary studies succeeded in providing a comprehensive view of the evolutionary processes of *C. angustisquama*. This series of investigations proposed multiple evolutionary precursors, strong genome homogenization, and lineage specific genetic effects as potential drivers to facilitate the adaptation to the extreme environments. In summary, the significance of my studies is that this is the first time to address the speciation processes via environmental adaptation in volcanic environments, contributing to our understanding of the formation processes of high plant diversity in Japan, a country of active volcanoes. In addition, my comparative genomics approaches shed light on the unknown possible mechanisms of low pH tolerance in plants. I believe that such a novel finding can be achieved because I have been focusing on the unique extremophyte in highly acidic solfatara fields.