

令和元年6月13日現在

機関番号：82105

研究種目：若手研究(A)

研究期間：2016～2018

課題番号：16H06197

研究課題名(和文) Investigating the resilience of Japan's cool climate forests to past and ongoing climate change

研究課題名(英文) Investigating the resilience of Japan's cool climate forests to past and ongoing climate change

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交付決定額(研究期間全体)：(直接経費) 16,700,000円

研究成果の概要(和文)：三年間で日本の固有2種ツガのために核伝子と葉緑体SSRマーカーを開発しました。2種とも全国で重要な遺伝子的構造が明らかにしました。特に南限と北限集団が遺伝子的距離が高かった。愛媛県の石鎚山で遺伝子マーカーを利用して一番南のコメツガ小集団を発見しました。SSRデータで記録せずRAD-seqデータがごく稀のコメツガ×ツガ過去雑種を発見しました。ブナに関しては全国に深く遺伝的に分布している葉緑体系統を明らかにしました。一つは西日本に分布しますが他の系統は東日本に分布しています。さらにブナの4ヶ所の瓢湖クラインのRAD-seqデータを獲得しました。解析まだ継続中です。

研究成果の学術的意義や社会的意義

Genetic isolation, hybridisation and competition with the temperate *T. sieboldii* threaten the long term viability of southern range-edge populations of the major Japanese subalpine tree *Tsuga diversifolia*.

研究成果の概要(英文)：Overall the study designed the first nuclear and chloroplast genetic markers for Japanese *Tsuga* species, the temperate *T. sieboldii* and subalpine *T. diversifolia*. Range-wide population genetic studies of both species found significant genetic structure with the most diverged populations being the southern and northern most occurrences in both species. Genetic markers also were used to discover the southernmost population of *T. diversifolia* at Mt Ishizuchi, Ehime Prefecture, which consists of a mixed stand of both species. Although not identified using SSRs, RAD-seq data found evidence for low levels of past hybridisation between the two *Tsuga* species in southern edge populations.

For *Fagus crenata*, whole chloroplast genome sequencing revealed two strongly diverged clades, one in western Japan that is closest to Chinese *Fagus* species and the other in eastern Japan with uncertain affinity. RAD-seq data for 354 samples from four elevational clines has been obtained and analysis is ongoing.

研究分野：樹木分子遺伝

キーワード：forest climate change genetic diversity hybridisation

様式 C - 19、F - 19 - 1、Z - 19、CK - 19 (共通)

1 . 研究開始当初の背景

Understanding how organisms will respond to climatic change is an increasingly urgent task given the pace of global warming. Indeed, impacts on forest ecosystems worldwide are already evident with tree dieback, regeneration failure and shifting vegetation communities recorded across the world. Climate change threatens not only to cause changes in the species composition and structure of forests but also the ecosystem services that they provide. Some of the most vulnerable forests are cool climate forests, especially where they grow at the warm-edge of their range. In the last 100 years annual mean temperature has risen by 1.1°C in Japan and future climate change is forecast to expose the forests of Japan to climates in the order of 2-3°C warmer by 2100 with a greater number of extreme hot days. Japan's cool climate forests are considered most at risk of decline under warming climates in western Japan where these forests are restricted to near the tops of relatively low mountain ranges with, for example, *Tsuga diversifolia* projected to lose 70% of suitable habitat by 2100 increasing the chance of extinction via not only physiological limits but competition/ hybridization with warm adapted congeneric species. However, cool climate forests of Japan have been subjected to multiple episodes of warming over the Quaternary (the last 2.5 million years) with, for example, the mid-Holocene thermal maximum around 6000 years ago being at least two degrees warmer than present. How and where cool climate forest species persisted during such times is therefore crucial to understanding how these forests will respond under future warming.

2 . 研究の目的

This study aimed to provide fundamental insights into the vulnerability of Japan's cool climate forests to climate change. To achieve this I: (1) examined whether isolated range-edge populations of three cool climate forest tree species, *Fagus crenata*, *Thuja standishii* and *Tsuga diversifolia*, withstood past warmer climates *in situ*; (2) understand whether hybridisation with *Tsuga sieboldii* is a threatening process in small range-edge populations of the subalpine *Tsuga diversifolia*; and (3) reveal the stock of genetic diversity adaptive to global warming in natural populations of *F. crenata*. This knowledge will enhance our ability to manage these crucial forests for Japan under global warming.

3 . 研究の方法

The first component of the study involved sampling warm-edge populations of both *F. crenata* and *T. diversifolia*. For *F. crenata*, whole chloroplast genome sequencing was used to compare the genetic diversity and divergence of warm edge populations to the closest core range populations in southern Kyushu and northern Kanto. For *T. diversifolia* and *T. standishii*, microsatellites were developed and used to genotype populations across the species range including all populations at the warm edge in western Japan.

The second component of the study involved sampling both *T. diversifolia* and *T. sieboldii* from where the species ranges overlap in parts of Shikoku, the Kii Peninsula and central Japan and genotyping using microsatellites and also a subset were sequenced using RAD-seq. The last component of the study involved RAD-seq genotyping a total of 354 *F. crenata* samples collected from four elevational clines of between 600 to 1000 m in elevation. Using this data, the divergence of low elevation populations from higher elevation sites will be assessed and SNPs under putative selection for temperature will be examined within and between the 4 clines.

4 . 研究成果

- A preliminary analysis of whole chloroplast genome sequences of warm edge populations of *F. crenata* showed that the chloroplast variation is not monophyletic with two strongly diverged clades in western and eastern Japan and the chloroplast lineage of Chinese *Fagus* nested within this variation. The origin of the two clades in Japan requires further research before progress can be made on the warm edge populations study.

- A range-wide population genetic study of *Tsuga diversifolia* showed significant genetic structure ($F_{st} = 0.039$) with the most diverged and genetically depauperate populations at the southern edge in Shikoku and Kii Peninsula and also in the isolated northern populations of Tohoku. Preliminary analyses show that low levels of backcrossed hybrids were detected by nuclear SSR markers and RAD-seq. This study also identified a previously unknown population of *T. diversifolia* at Mt Ishizuchi, Ehime Prefecture; the most southern in the species.

- 15 nuclear microsatellites were developed for *T. standishii* and 20 populations collected from across the species range including all known population in western Japan.

5 . 主な発表論文等

[雑誌論文](計4件)

1. Worth, J.R.P., Luxian Liu, Fu-Jin Wei and Nobuhiro Tomaru. (2019) The complete chloroplast genome of *Fagus crenata* (subgenus *Fagus*) and comparison with *F. engleriana* (subgenus *Engleriana*). PeerJ 7:e7026 DOI 10.7717/peerj.7026, 10 pages. 査読有
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3. Liu L.X., Li P., Zhang H.W., & Worth, J.R.P. (2018). Whole chloroplast genome sequences of the Japanese hemlocks, *Tsuga diversifolia* and *T. sieboldii*, and development of chloroplast microsatellite markers applicable to East Asian *Tsuga*. *Journal of Forest Research*, 23: 318-323, 査読有
4. Worth, J. R.P., Yang J. C., Kanetani S., Kikuchi S., Sakaguchi S. & Ihara-Udino, T. (2018) Development of nuclear microsatellite markers for the Japanese conifers *Tsuga diversifolia* and *T. sieboldii* (Pinaceae). *Plant Species Biology* 33: 67-71, 査読有

〔学会発表〕(計 件)

〔図書〕(計 件)

〔産業財産権〕
出願状況(計 件)

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

取得状況(計 件)

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

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