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Historical seed use and transfer affects geographic specificity in genetic diversity and structure of old planted *Pinus thunbergii* populations

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ABSTRACT

Although most molecular ecology studies examining genetic variation have focused on natural forests, for some major tree species, natural forests are nearly extinct, and the remaining genetic resources are mainly planted forests. In order to manage the genetic variability and develop a conservation strategy for such species, it is important to examine genetic variation resulting from historical processes during repeated artificial population development through plantations. The geographic pattern of genetic diversity and structure of 49 old planted *Pinus thunbergii* populations (2755 trees) distributed across Japan was examined using seven nuclear microsatellite markers. We found that allelic diversity was generally lower in both northern and eastern populations; however, locally, some populations in other regions also exhibited low allelic diversity. The overall value of the standardized measure of population differentiation ($G'_{ST} = 0.206$) was higher than that of both other widespread Japanese conifers and continental *Pinus* species. STRUCTURE software revealed a general gradual cline in genetic structure from southwestern to northeastern populations; however, some populations on the Japan Sea side showed quite a different local proportion of cluster memberships from nearby populations. These observations indicated that most of the preserved, planted populations of *P. thunbergii* possess regional genetic variation, but some populations were developed from seed pools derived from other regions, possibly through distribution by ship along the Japan Sea. Information on this specific genetic variation as a result of historical seed use and transfer should assist the design of several conservation units and breeding zones, while also taking care of a deep-seated need for conservation of pine forests by local people.

1. Introduction

Evaluation of the genetic variation of forest trees across their distribution range provides fundamental information required for conservation of regional forest ecosystems and genetic resource management, especially for major tree species with a wide distribution range and with high ecological and economic value (e.g., Tsuda and Ide, 2005; Tsumura et al., 2007a; Sutherland et al., 2010; Iwaizumi et al., 2013a). Most molecular ecology studies examining the genetic variation of major tree species have focused on natural forests, with the main objective of understanding the species' genetic dynamics affected by phylogeographical processes (in *Pinus*: e.g., Karhu et al., 1996; Richardson et al., 2002; Gonzalez-Martinez et al., 2004; Boys et al., 2005; in Japanese forest tree species: e.g., Takahashi et al., 2005; Tsuda and Ide, 2005; Hiraoka and Tomaru, 2009). However, for some major species, natural forests have become almost extinct and the main genetic resources are limited to planted forests. Genetic composition of planted forests may have been artificially influenced by historical economic, social and cultural human activities through seed collection and transfer, planting of seedlings, and population redevelopment and maintenance (e.g., Izuno et al., 2013; Win et al., 2016). In order to monitor and manage genetic variability and develop a conservation strategy for such tree species, it is particularly important to examine how the genetic pattern of the remaining planted forests of the species differs from that of species in natural forests, and to consider how their genetic variation relies on historical processes.

Pinus thunbergii Parl (Japanese black pine) is a widely distributed conifer throughout Honshu, Shikoku and Kyushu (including Yaku Island) in Japan (see Fig.1). The natural forests of the species were frequently distributed in coastal areas, but the species has often also

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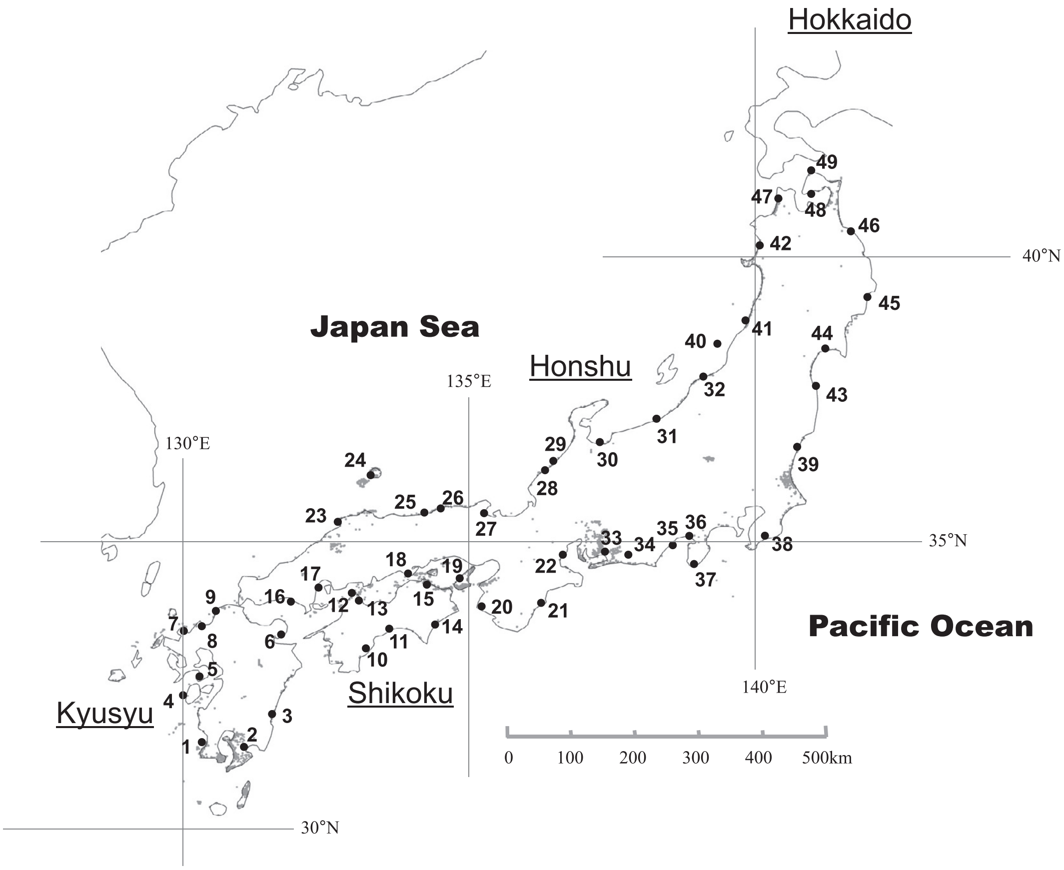

Fig. 1. Locations of the 49 investigated populations of *Pinus thunbergii*. Gray areas represent the present distribution range of *P. thunbergii*, generated at a spatial resolution of ca. 1 km^2, based on vegetation data from the 5th National Survey on the Natural Environment by the Ministry of the Environment, Japan (National Conservation Bureau, Ministry of the Environment 2004). Numbers correspond to the population numbers in Table 1. The longitudinal scale based on the distance at 32°N is also represented.

been planted in coastal areas to prevent land erosion and provide protection from wind-blown sand and tidal waves, contributing to the expansion of agriculture in inland plains. The species is also ecologically important and its dominance in coastal forests plays a role in maintaining these ecosystems. Moreover, *P. thunbergii* forests have social and cultural importance; old planted forests in many areas of Japan, which have been maintained by the local people, are a place of traditional picturesque or relaxing scenery, constituting a part of Japanese historical coastal landscapes. However, introduction of the pine wood nematode (*Bursaphelenchus xylophilus*) from North America resulted in a marked irreversible decline of most natural *P. thunbergii* forests in Japan in the past 40–50 years (Mamiya, 1988), and currently, resources of *P. thunbergii* are limited primarily to the above old planted populations that have been strictly protected. It is important to consider the species' appropriate several units for conserving genetic resources ('conservation units') in order to maintain the current local genetic variation remaining in these old plantations, and several zones for breeding programs ('breeding zones') in order to avoid genetic disturbance and/or reforestation failure and utilize breeding materials (defined according to the review of Westfall and Conkle, 1992).

The artificial processes influencing establishment of old planted *P. thunbergii* populations may have resulted in a geographically unusual genetic structure, a pattern somewhat different from that of natural populations. Because of the multiple roles of *P. thunbergii* forests, it is believed that, since around the 17th century, new coastal pine forests have sometimes been established from seedling plantations derived from seed sources from other regions and from seed transfer over long distances. As a result of seed introduction from other regions, the introduced population could show different genetic variation from neighboring populations. During long-term repeated development of the population by the collection of seeds and planting of seedlings, certain regions or populations could suffer from reduction in genetic diversity or increased genetic fixation due to genetic bottlenecks or drift (e.g., Hamrick et al., 1992; Suyama et al., 1997; Takahashi et al., 2000). By detecting genetic patterns peculiar to planted forests, the actual historical background of the species can be inferred. Genetic variation of *P. thunbergii* was investigated for natural populations 30 years ago using allozyme markers (Miyata and Ubukata, 1994); however, most of these populations have become extinct because of further attacks by pine wood nematodes. Genetic reevaluation of old planted populations of the species, based on higher resolution DNA markers, is needed to examine differences from past natural populations and to understand the factors with genetic consequences.

The present study examined the current geographic pattern of genetic diversity and population structure of old *P. thunbergii* plantations across Japan using nuclear microsatellite (also known as simple sequence repeat or SSR) markers. We discuss the possible factors for reconstructing the genetic variability of this species in light of the historical artificial processes affecting it, in order to design programs for conservation and forest management.

2. Materials and methods

2.1. Sample collection and DNA analysis

Mature needles were collected from 2755 *Pinus thunbergii* trees representing 49 planted populations (Table 1). All of these populations are old stands (at least 80 years old) that have been maintained by local people as representative regional resources of this species, most of which have been authorized among the top 100 coastal pine forests of