Diversity and Conservation of Genetic Resources of an Endangered Five-Needle Pine Species, *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima

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Abstract—*Pinus armandii* var. *amamiana* is endemic to two small islands in the southern region of Japan and listed as a vulnerable species. The large size and high wood quality of the species have caused extensive harvesting, resulting in small population size and isolated solitary trees. Genetic diversity of the *P. armandii* complex was studied using allozyme analyses. Genetic distances between (1) *P. armandii* var. *amamiana* and *P. armandii* var. *armandii* and (2) *P. armandii* var. *amamiana* and *P. armandii* var. *mastersiana* were 0.488 and 0.238, respectively. These genetic differences were comparable with congeneric species level (0.4) and much greater than conspecific population level (less than 0.1) that occur in *Pinus* species in general. No differences in diversity were recognized between populations of *P. armandii* var. *amamiana* from each island. The impact of human activity on the endangered status of *P. armandii* var. *amamiana* in Tane-ga-shima Island was demonstrated by inspecting historical records, starting in the 16th century. A strategy for conservation of *P. armandii* var. *amamiana* was discussed in consideration of sparse distribution, pollen flow, and the effects of pine wilt disease, caused by *Bursaphelenchus xylophilus*.

Key words: *Pinus armandii* var. *amamiana*, endangered species, genetic diversity, pollen flow, conservation strategy

Introduction

Pinus armandii Franch. var. *amamiana* (Koidz.) Hatusima, is an endangered pine species endemic to Tane-ga-shima and Yaku-shima Islands, southern Japan (Yahara and others 1987). The species is closely related to *P. armandii* var. *armandii* that is distributed in the western part of continental China and *P. armandii* var. *mastersiana* Hayata from the highlands of Taiwan. The wood of *P. armandii* var. *amamiana* was traditionally used for making fishing canoes and also used in house construction (Kanetani and others 2001). Consequently, large numbers of *P. armandii* var. *amamiana* trees have been harvested and populations have dwindled on both islands. Currently, the estimated number of surviving *P. armandii* var. *amamiana* trees in natural populations are 100 and 1,000 to 1,500 on Tane-ga-shima and Yaku-shima Islands, respectively (Yamamoto and Akasi 1994).

In recent years, the number of *P. armandii* var. *amamiana* trees has rapidly declined, with dead trees frequently observed (Hayashi 1988; Yamamoto and Akashi 1994; Kanetani and others 2002). Several factors are responsible for the recent decline, including inbreeding depression (Hayashi 1988; Kanazashi and others 1998), reduced natural regeneration (Chigira 1995; Kanetani and others 1998), and pine wilt disease (Hayashi 1988; Yamamoto and Akashi 1994; Nakamura and others 2002). *Pinus armandii* var. *amamiana* has been classified as an "Endangered" species in the *Japanese Red List*, a compilation of endangered Japanese species, due to the rapid decrease in population size and the isolation of small populations (Environment Agency of Japan 2000).

In order to establish an *in situ* conservation scheme for an endangered species, it is important to collect information on the species in natural habitats, such as decline and genetic variation (compare Primack 1995; Meffe and Carroll 1997). In this study, we clarified the genetic diversity and phylogenetic relationship of *P. armandii* var. *amamiana* with other *P. armandii* varities, researched the historical distribution of the species on Tane-ga-shima Island, and propose a strategy to conserve the genetic resources of this species.

Materials and Methods

Study Site

Tane-ga-shima and Yaku-shima Islands are located about 60 km south of Kyushu Island in southern Japan (fig.1). These islands are approximately 20 km apart. Tane-ga-shima Island is relatively flat (elevation to 282 m), 58 km long (north to south), and 10 km wide (west to east). In contrast, Yaku-shima Island is round with a diameter of about 30 km and dominated by a series of peaks over 1,800 m in height. About 21 percent of the island has been registered as a World Natural Heritage Area in 1993.

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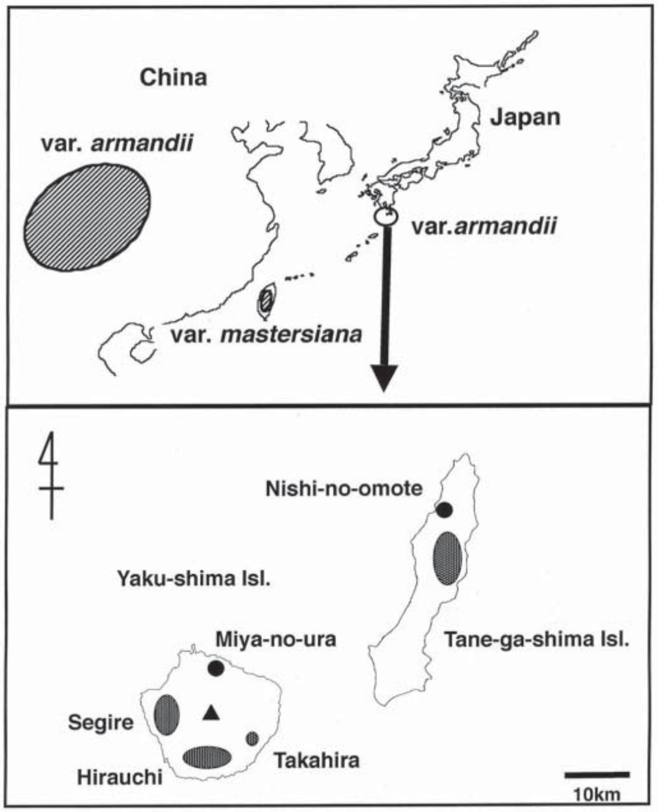

Figure 1—Distribution of *Pinus armandii* vars. *amamiana*, *armandii* and *mastersiana* populations.

Surviving *P. armandii* var. *amamiana* trees are located in the center of Tane-ga-shima Island, while three local populations of this species are distributed from 300 to 800 m elevation on Yaku-shima Island (Yamamoto and Akashi 1994; Kanetani and others 1997).

Genetic Variation

We collected 88 samples of *P. armandii* var. *amamiana* from three populations, Segire, Takahira and Hirauchi, on Yaku-shima Island, and 17 samples from Tane-ga-shima Island. The trees from Tane-ga-shima Island were treated as one population because most trees are solitary or occur in small groups. We collected 24 samples of *P. armandii* var. *armandii* from one natural population near Dali, Yunnan, China, and 12 samples of *P. armandii* var. *mastersiana* from seedlings originating from a population near Musha, Taiwan.

Crude extracts from inner bark of twigs were prepared according to Yahara and others (1989) for allozyme analyses. The starch gel electrophoretic system with tris-borate buffer (#8 in Soltis and others 1983) was used for allozyme analysis on mannose phosphate isomerase (*MPI*; 1), phosphoglucoisomerase (*PGI*; 2) and triose-phosphate isomerase (*TPI*; 2). The numbers in parentheses refer to the numbers of allozyme loci employed in the following analyses. Another starch gel electrophretic system with histidine and citric acid buffer (Cardy and others 1981) was used for isocitrate dehydrogenase (*IDH*; 1), malate dehydrogenase (*MDH*; 2), 6-phosphogluconate dehydrogenase (*6PGD*; 1), phosphoglucomutase (*PGM*; 2), shikimate dehydrogenase (*SkDH*; 1) and UDP glucose pyrophosphorylase (*UGP*; 1). Acrylamide gel electrophoresis (Tsumura and others 1990) were used for asparatate aminotransferase (*AAT*; 2), amylase (*AMY*; 1), esterase (*EST*; 1), glutamate dehydrogenase (*GDH*; 1) and leucine aminopeptidase (*LAP*; 2).

Calculations of Genetic Parameters

Three kinds of genetic diversity (*P*, rate of polymorphic loci; *A*, mean number of alleles; *He*, expected mean heterozygosity) and fixation index for the populations of *P. armandii* var. *amamiana* were calculated. For calculations of Nei's genetic distance and genetic identity (Nei 1972), the Segire population was excluded, since the number of examined loci was different from those of the other populations. F-tests were used to detect significant differences.

Historical Distribution of *P. armandii* var. *amamiana* on Tane-ga-shima Island

We surveyed historical records owned by Kagoshima Prefectural Museum of Culture, Reimei-kan for all descriptions on surviving number and size of "Goyo-matsu (5-needle-pine; var. *amamiana*)" and the number of "Maruki-bune" (canoes) constructed.

Results

Genetic Variation

The three populations on Yaku-shima Island showed values of 0.069-0.131 (mean: 0.100) for *He*, while the *He* value for the Tane-ga-shima population was 0.112 (table 1). Although the population of surviving trees in Tane-ga-shima Island is much less than populations on Yaku-shima Island, it contains the nearly same amount of genetic diversity. The Fixation Index (*F*) in the Tane-ga-shima population, 0.198 (not significant), is larger than those of three populations in Yaku-shima Island, 0.012-0.074 (mean: 0.051).

Genetic identity and Nei's standard genetic distance among the populations of *P. armandii* vars. *amamiana*, *armandii*, and *mastersiana* were shown in table 2. Mean genetic distance among *P. armandii* var. *amamiana* populations was ranged from 0.003 to 0.029 (mean: 0.017). Genetic distances between *P. armandii* var. *amamiana* vs. var. *mastersiana*, *P. armandii* var. *amamiana* vs. var. *armandii* and *P. armandii* var. *armandii* vs. var. *mastersiana* were 0.460-0.510 (mean: 0.488), 0.220-0.250 (mean: 0.238) and 0.137, respectively.

Historical Distribution on Tane-ga-shima Island

In Tane-ga-shima Island, harvest of large *P. armandii* var. *amamiana* populations was regulated by the Shimadzu local government from the 16th through the 19th century (Kanetani and others 2001). Descriptions of the number and stem girth of *P. armandii* var. *amamiana* trees were found from 1685 to 1782. In particularly, 428 *P. armandii* var. *amamiana* trees with stem girth above 150 cm were recorded in 1755. Table 3 is a summary of the number of

Table 1—Genetic diversity and fixation index of *Pinus armandii* var. *amamiana* populations.

Population	Number of loci	*P*[a]	*A*[b]	*He*[c]	*F*[d]
Segire (Yaku-shima Island)	13	0.31	1.31	0.069	0.067[ns]
Takahira (Yaku-shima Island)	20	0.50	1.60	0.131	0.074[ns]
Hirauchi (Yaku-shima Island)	20	0.40	1.45	0.101	0.012[ns]
Tane-ga-shima Island	20	0.45	1.55	0.112	0.198[ns]

a: rate of polymorphic loci
b: mean number of alleles per locus
c: mean heterozygosity
d: fixation index
ns: not significantly different from zero

Table 2—Genetic identity (upper triangle) and Nei's standard genetic distance (lower triangle) among *Pinus armandii* varieties.

Population (variety)	1	2	3	4	5
1. Takahira (var. *amamiana*)		.980	.998	.631	.803
2. Hirauchi (var. *amamiana*)	.020		.971	.600	.779
3. Tane-ga-shima Island (var. *amamiana*)	.003	.029		.610	.784
4. Dali, Yunnan, China (var. *armandii*)	.460	.510	.495		.872
5. Musha, Taiwan (var. *mastersiana*)	.220	.250	.244	.137	

Table 3—Historical record of stem girth and number of trees of *Pinus armandii* var. *amamiana* growing on Tane-ga-shima Island.

Year	Stem girth (cm)	Number of trees
1685	—	247
1748	210 - 420	355
1755	210 - 420	218
	150 - 180	210
1782	—	28

trees and stem girth of *P. armandii* var. *amamiana* found in the historical record.

It is known large numbers of *P. armandii* var. *amamiana* were harvested for making fishing canoes and house construction during late 19th and early 20th century (Kanetani and others 2001). In 1918, 455 canoes were made probably from *P. armandii* var. *amamiana*. Canoes were used for fishing until twenty years ago in Tane-ga-shima Island.

Discussion

The genetic diversity level of *P. armandii* var. *amamiana* (*He*: 0.069-0.131) is a little lower than the mean value of the genus *Pinus* (*He*: 0.136) (Hamrick and others 1992), in which Hamrick and Godt (1996) recognized wide variation of genetic diversity and population structure. In comparison, *Pinus torrayana*, an endemic species in California with an extremely restricted distribution, has a low genetic diversity of 0.017 (Ledig and Conkle 1983). *P. armandii* var. *amamiana* also has a limited distribution, but maintains a more or less high genetic diversity level. In Tane-ga-shima Island, the trees are separated each other but still retain a level of genetic diversity as populations on Yaku-shima Island. This indicates that the serious decrease of tree number in Tane-ga-shima Island occurred in the near past. Fixation indices are almost zero in Yaku-shima Island populations and 0.198 in Tane-ga-shima Island. This value shows that adult *P. armandii* var. *amamiana* trees in Yaku-shima Island have been produced from random mating. The high value in Tane-ga-shima Island may be explained by the Wahlund effect.

The genetic difference among the three conspecific taxa of *P. armandii* is large. Hamrick and Godt (1996) reviewed genetic heterogeneity of pine populations and introduced some species with disjunct distributions that have high gene diversity levels. The genetic difference of the varieties in *P. armandii* is at the congeneric species level (0.4) and much greater than conspecific population level (less than 0.1) according to Gottlieb (1977, 1981) and Crawford (1983). Therefore genetic conservation on *P. armandii* should be conducted at least for varieties *amamiana*, *armandii* and *mastersiana*, respectively.

A bibliographical study demonstrated the destructive cuttings of *P. armandii* var. *amamiana* in Tane-ga-shima Island until the early 20th century. The species was preferred for making canoes because of a greater amount of resin than in other tree species in Tane-ga-shima Island and only large trees, 2.7m - 6.3 m were used. The historical record indicates that 400 trees were preserved about 250 years ago (table 3). In 1918, however, harvesting must have increased, as 455 canoes made of *P. armandii* var. *amamiana*, were counted. Recently, we have discovered that a few *P. armandii* var. *amamiana* trees were harvested for use in quarries (Kanetani and others 2001). Therefore, the human impact on this species is still continuing.

As a consequent of human impacts, the reproductive potential of the surviving trees on Tane-ga-shima Island may be limited. Kanazashi and others (1998) and Nakashima and Kanazashi (2000) compared cone yields from artificial pollinations to natural (open) pollinations on several isolated trees on Tane-ga-shima Island. The percentage of filled seeds per cone from natural pollinations (15.4 to 37.7 percent) was less than artificial cross-pollinations, which ranged from 66.8 to 97.3 percent. However, there was no significant difference (F-test) in the percentage of filled seeds after artificial self-pollination (34.3 percent) and after open-pollination (34.6 percent). This suggests that natural pollination among Tane-ga-shima Island trees could be primarily self-pollination, which will promote inbreeding depression.

P. armandii var. *amamiana* are now threatened by pine wilt disease, an epidemic disease of the genus *Pinus* in Japan caused by the nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Kiyohara and Tokushige 1971; Kishi 1995). This disease has been inferred to be a major mortality factor of *P. armandii* var. *amamiana* in natural populations (Hayashi 1988; Yamamoto and Akashi 1994). Recently, the nematode's presence was confirmed through detection in dead *P. armandii* var. *amamiana* trees in Tane-ga-shima Island (Nakamura and others 2002). Pine wilt disease, therefore, is a significant threat to the continued existence of natural populations of *P. armandii* var. *amamiana*.

The serious decline of *P. armandii* var. *amamiana* necessitates the formation of a strategy for genetic conservation. Protection and management should be required for *in situ* populations on both islands, and demise from pine wilt disease should be carefully monitored. The small, diffuse population on Tane-ga-shima Island requires artificial cross pollinations for restoration of seed fertility and successful reproduction. Additionally, the establishment of *ex situ* plantations containing grafts of mature trees of *P. armandii* var. *amamiana* (for *ex situ* conservation) is needed to ensure conservation of genetic diversity of Tane-ga-shima Island populations.

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