日本産タイキンギクにおける酵素多型遺伝子座の単型性: 台湾の集団と比較して

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Masayuki Maki[1], Goro Kokubugata[2] and Tadashi Yamashiro[3]: **Lack of allozyme diversity in populations of the endangered perennial *Senecio scandens* (Asteraceae) in Japan: comparison with a population in Taiwan**

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Genetic diversity is considered to be one of the critical factors determining population/species preservation (Frankham et al. 2002). Enzyme electrophoresis is a conventional method for screening genetic diversity. This technology is straightforward compared to other techniques for identifying molecular markers, and has resulted in the accumulation of a vast amount of information facilitating comparisons among the data.

In general, allozyme diversity is lower in rare and/or geographically limited species than in common and widespread ones (Karron 1987; Hamrick and Godt 1995; Cole 2003), but this is not always the case (Gitzendanner and Soltis 2000; Maki 2003). Some studies revealed no allozyme diversity in rare and endemic species (Waller et al. 1987; McLeod and Reilly 2004; Chung 2009).

Senecio scandens Buch.-Ham. ex D. Don is a perennial herb that is widely distributed from Nepal to Japan, via south China and Taiwan (Kitamura 1991; Koyama 1995). In Japan, this species is limited to the southern parts of the Kii Peninsula and Shikoku; however it occasionally builds up large populations in these areas. The plants mainly occur near the seashore, but are sometimes seen in woodland margins. The Red Data Book of Japanese plants (Environment Agency of Japan 2007) listed *S. scandens* as VU (vulnerable to extinction), following the International Union for Conservation of Nature (IUCN) criteria (1994). The somatic chromosome number is 2n=20, suggesting that the species is diploid. The existence of the species is threatened by road construction and land reclamation (Environment Agency of Japan 2007).

In this study, we examined the allozyme diversity in a total of 16 populations of *S. scandens* in Japan, and in a population in Taiwan for comparison.

Materials and Methods

Sampling

A mature leaf was sampled from 25 randomly selected individuals from the populations in Japan (J1–J16 in Fig. 1). Because *S. scandens* propagates by rhizomes, leaves were collected at meter intervals to minimize the chance of collecting multiple samples from the same genet. Nineteen individuals were sampled in a similar manner from the population in Taiwan (T1 in Fig. 1).

Allozyme electrophoresis and data analyses

Enzyme extraction and polyacrylamide gel electrophoresis were carried out following the method of Maki and Murata (2001). Eleven enzyme systems were examined: alcohol dehydrogenase (ADH; EC 1.1.1.1), aspartate amino transferase (AAT; EC 2.6.1.1), formate dehydrogenase (FDH; EC 1.2.1.2), fructose-bisphosphate aldolase (ALD; EC 4.1.2.13), glutamate dehydrogenase (GDH; EC 2.7.1.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), leucine aminopeptidase (LAP; EC 3.4.11.1), menazion reductase (MNR; EC 1.6.99), phosphoglucomutase (PGM; EC 5.4.2.2), superoxide dismutase (SOD; EC 1.15.1.1), and triose-phosphate isomerase (TPI; EC 5.3.1.1). The staining protocols are described in Tsumura et al. (1990), except for that of FDH, which followed Wendel and Weeden (1989).

All individuals were genotyped at the al-

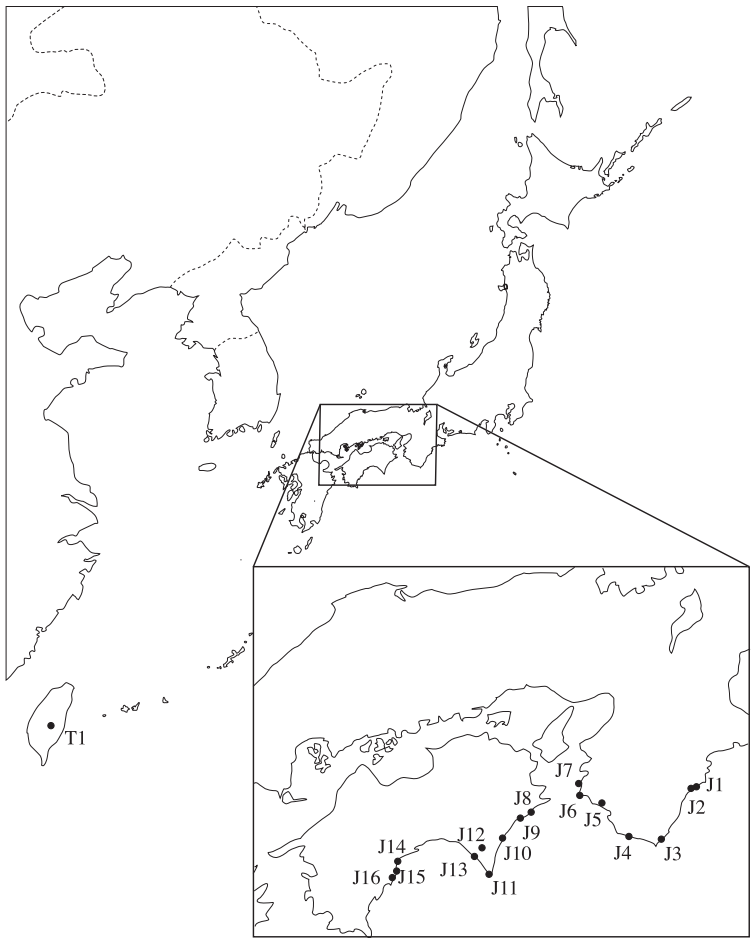

Fig. 1. Populations of *Senecio scandens* examined in this study.

lozyme loci, and allele frequencies were calculated based on the genotypes for the populations. The standard genetic identity (*I*) between the populations in Japan and Taiwan (Nei 1972) was calculated.

Results and Discussion

A total of 18 putative loci were detected in the 11 enzyme systems examined in this study: *Adh, Aat-1, Aat-2, Aat-3, Ald, Fdh-1, Fdh-2, Gdh, Gpi, Lap, Mnr-1, Mnr-2, Mnr-3, Pgm-1, Pgm-2, Sod-1, Sod-2*, and *Tpi*. The number of the loci detected in each enzyme did not indicate any evidence of duplications in the loci and suggested that the species is diploid (Gottlieb 1982; Weeden and Wendel 1989).

All the enzyme loci were monomorphic in the populations examined in Japan (Table 1). In contrast, the population in Taiwan (T1) was considerably polymorphic for eight loci. The percentage of polymorphic loci (*P*), the number of alleles per locus (*A*), and the expected heterozygosity (*h*) were 61.1, 1.56, and 0.146, respectively.

Nei's genetic identity (Nei 1972) between the populations in Japan and Taiwan was substantially low (*I*=0.699), suggesting that these populations are genetically differentiated at the species level rather than at the population level (Gottlieb 1981). Kitamura (1991) observed many individuals of *S. scandens* in Taiwan with lobed leaves and regarded such types as the forma, *S. scandens* f. *incisus* (Franchet) Kitamura. Kitamura (1991) noted that there are some specimens with lobed leaves from regions other than Japan although those in the Himalayas of the type locality do not have lobed leaves. There were no other morphological dif-

Table 1. Allele frequencies in the 18 putative enzyme loci in the Japan's and Taiwan's populations of *Senecio scandens*

Locus	allele	Japan J1-J16	Taiwan T1
Adh	a	0.000	0.052
	b	1.000	0.895
	c	0.000	0.052
Aat-1	a	0.000	1.000
	b	1.000	0.000
Aat-2	a	0.000	1.000
	b	1.000	0.000
Aat-3	a	0.000	0.052
	b	1.000	0.948
Ald	a	1.000	1.000
Fdh-1	a	1.000	1.000
Fdh-2	a	1.000	1.000
Gdh	a	1.000	0.658
	b	0.000	0.342
Gpi	a	0.000	0.473
	b	1.000	0.000
	c	0.000	0.537
Lap	a	0.000	0.158
	b	1.000	0.000
	c	0.000	0.842
Mnr-1	a	0.000	0.105
	b	1.000	0.895
Mnr-2	a	1.000	1.000
Mnr-3	a	0.000	0.333
	b	0.000	0.300
	c	1.000	0.367
Pgm-1	a	1.000	0.000
	b	0.000	1.000
Pgm-2	a	1.000	1.000
Sod-1	a	0.000	0.052
	b	1.000	0.948
Sod-2	a	1.000	1.000
Tpi	a	1.000	1.000

ferences between *S. scandens* from Japan and Taiwan. In our study, most of the Japanese populations were located near the seashore, while those from Taiwan were in an understory along a valley in a mountain area, indicating that the plants in the two areas have different ecological preferences.

There are two possible explanations for the genetic paucity of *S. scandens* in Japan. In the first scenario we propose that *S. scandens* had a wider and more continuous distribution in the past, but a range contraction and subsequent expansion occurred due to climate change. In the glacial era, plant species preferring mild climates may have sought refuge in warmer areas, i.e., refugia, where they maintained narrow distributions; also, some refugia were located in the Japanese Archipelago (Tsukada 1983). A bottleneck effect occurring in the refugia could have resulted in the loss of most genetic diversities which have not been recovered in *S. scandens* in Japan due to a relatively low mutation rate in the allozyme loci.

In the second scenario we propose that the populations in Japan may have been established as a result of long-distance seed dispersal from adjacent areas. In this case, the founder effect is considered to be a major cause of the lack of allozyme diversity in the Japanese populations. The pappus of the mature fruit of *S. scandens* may enable the seed to be carried some distance. However, because of the large distance between the present distribution of *S. scandens* in Japan and the nearest other populations, this scenario appears to be the less plausible of the two. In either case, the timing of the vicariance event was far enough in the past to cause large genetic differentiation between the populations in Japan and Taiwan and adaptation to different habitats.

Phylogeographical studies on *S. scandens* using other molecular markers and more populations from a wider geographical range may be useful to elucidate the causes of the genetic depauperation of *S. scandens* in Japan.

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牧　雅之[1]・國府方吾郎[2]・山城　考[3]：日本産タイキンギクにおける酵素多型遺伝子座の単型性―台湾の集団と比較して

キク科タイキンギクは，ネパールから中国南部，台湾を経て日本まで広く分布する多年生草本である。国内では，紀伊半島南部と四国南部に限られて分布し，レッドデータブックでは近年の道路建設や土地造成のために絶滅危惧 II 類として記載されている。本研究では，国内の 16 集団と比較のために台湾の 1 集団をサンプリングし，電気泳動を行って，11 の酵素種における 18 個の酵素多型遺伝子座を検出した。

国内の 16 集団はすべて同じ対立遺伝子に固定しており，18 個の酵素多型遺伝子座について変異は全く見られなかった。一方，台湾の集団は多型性がかなり高かった。国内の集団と台湾の集団の間の遺伝的同一度は 0.699 となり，種内レベルとしては低い値を示した。

国内の集団で遺伝的多型が見られなかった理由としては，2 つの理由が考えられる。一つは，タイキンギクは過去にもっと広く連続的に分布していたが，過去の寒冷期に温暖なレフュージアに閉じこめられ，その際にビン首効果により遺伝的多様性を失った可能性がある。もう一つは，近隣の地域からの（おそらくは台湾からの）長距離散布により分布を拡大し，その際の創始者効果を受けた可能性である。日本の分布域と他の分布域の間に集団が全く見つからないことから，後者の可能性は前者より低いと思われる。今後，別の分子マーカーを用いて，他地域の集団を含めて解析することにより，日本の集団における遺伝的多様性の減少を明らかにできると考えられる。

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