

# 日本産高山植物における葉緑体DNAの種内変異

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# Noriyuki Fujii\*, Kunihiro Ueda\*\* and Tatemi Shimizu\*\* : Intra-specific Sequence Variation of Chloroplast DNA in Japanese Alpine Plants

藤井紀行\*・植田邦彦\*\*・清水建美\*\* : 日本産高山植物における葉緑体DNAの種内変異

We reported on the intraspecific sequence variation of *Primula cuneifolia* Ledeb. (Primulaceae) and *Pedicularis chamissonis* Steven (Scrophulariaceae) using the non-coding region of chloroplast genome (Fujii *et al.* 1995, Fujii *et al.* unpublished). These species are distributed in the North Pacific coastal area from the Japanese Archipelago northeastwards to southwest Alaska, and occur in the subalpine and alpine regions in Japan. In both species, many haplotypes of chloroplast DNA (cpDNA) could be recognized, and each haplotype was found to be geographically structured. Furthermore, we could obtain the phylogenetic information among the haplotypes of these species.

In order to examine the magnitude of intraspecific cpDNA variation of other plants occurring in the subalpine and alpine areas in Japan, we shall try to survey 40 taxa using the nucleotide sequences of non-coding regions of cpDNA.

## Materials and Methods

We selected 40 taxa at random from subalpine and alpine plants in Japan (Table 1). Four species groups were included there: species in *Taraxacum* F. H. Wigg., *Leontopodium* R. Br. ex Cass. (Asteraceae), *Coelopleurum* Ledeb. (Umbelliferae), and *Veronica stelleri* Pall. ex Link and *V. nipponica* Makino ex Matsum. (Scrophulariaceae) group, which have been considered to be closely related one another, respectively. In 36 species and four species groups, we collected a total of two to 29 plants from two to 26 populations. The taxonomy of all species was followed by Shimizu (1982a, 1983), except for *Oxalis acetosella* L. (Oxalidaceae) (Shimizu 1982b).

Collected leaves were dried by silica gel powder in the fields and transported to the laboratory. Total DNAs were extracted from the dried leaves following a modification of the CTAB method of Doyle and Dickson (1987). The intergenic spacer between *trnL* (UAA) 3'exon and *trnF* (GAA) of cpDNA was amplified using polymerase chain reaction (PCR) technique in all accessions. We also amplified the *trnL* (UAA) intron in a part of samples of *Solidago virga-aurea* L. (Asteraceae), *Vaccinium vitis-idaea* L. (Ericaceae), and *Epilobium angustifolium* L. (Onagraceae). Sequences of the two pairs of primers for PCR and sequencing are as follows; the intergenic spacer: 5'-GGTTCAAGTCCCTCTATCCC-3' and 5'-ATTTGAACTGGTGACACGAG-3', the intron: 5'-CGAAATCGGTAGACGCTACG-3' and 5'-GGGATAGAGGGACTTGAAC-3' (Taberlet *et al.* 1991). The PCR products excised from agarose gel were purified using the GENE-CLEAN II Kit (BIO 101). Obtained DNAs were sequenced by the A.L.F. II autosequencing machine (Pharmacia) using the AutoSequencer Core Kit (TOYOBO) or CircumVent Sequencing Kit (New England Biolabs). In the intergenic spacer, about a half of taxa were sequenced in a single direction using forward primer on *trnL* (UAA) 3'exon.

Sequence data were aligned manually with the DNASIS-Mac (Hitachi Software Engineering). Insertions/deletions (indels) were generally placed so as to increase the number of matching nucleotides in a sequence position. Then, we determined the cpDNA haplotypes of all samples from site-change data and indels.

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### Results and Discussion

The lengths of the intergenic spacer between *trnL* and *trnF*, and *trnL* intron were variable according to taxa (Table 1). Among all accessions, the lengths of the intergenic spacer varied from 158 bp (*Oxytropis nigrescens* Fisch., Leguminosae) to 449 bp (*Geum calthifolium* Sm. var. *nipponicum* (F. Bolle) Ohwi, Rosaceae), and those of the intron varied from 450 bp (*Solidago virga-aurea*) to 550 bp (*Epilobium angustifolium*). The member of Leguminosae had short length of the intergenic spacer, especially.

In 19 taxa (16 species and three species groups), there was not observed neither intra- nor interspecific cpDNA variation at all. For example, there were no molecular polymorphisms in *Orchis aristata* Fisch. (Orchidaceae) among 13 populations from Mt. Kitadake of central Honshu in Japan to Unalaska Island of the Aleutians. Although the *Leontopodium* species, *L. discolor* P. Beauv. and *L. hayachinense* (Takeda) H. Hara et Kitam., have been recognized as different species each other from the external morphology, the sequences of the intergenic spacer in both species were identical.

Intra- and interspecific variations of cpDNA were detected in 21 taxa (20 species and one species group). The taxa that were found to possess relatively many polymorphic characters were as follows: *Taraxacum* species, *Veronica schmidtiana* Regel ex F. Schmidt, *Pedicularis yezoensis* Maxim. and *P. verticillata* L. (Scrophulariaceae), *Gentiana nipponica* Maxim. (Gentianaceae) and *Trollius riederianus* Fisch. et C. A. Mey. (Ranunculaceae). The maximum values of the proportion of mutational events (O'Donnell 1992) of these taxa were 0.018, 0.020, 0.013, 0.035, 0.016 and 0.010, respectively. According to our previous studies, the value was 0.019 in *Primula cuneifolia* (Fujii *et al.* 1995), and that was 0.021 in *Pedicularis chamissonis* (Fujii *et al.* unpublished). In the above six taxa, therefore, we may be able to clarify the phylogenetic relationships among the cpDNA haplotypes by adding another sequence data. *P. verticillata* showed the highest value (0.035) among all taxa. This was found in the haplotype of the population of Mts. Hakusan in Honshu, Japan (Type C), and it had many more polymor-

phic characters (8 bp nucleotide substitutions and three indels against Type A) than other haplotypes. In external morphology, the plants of the population of Mts. Hakusan are more slender than the others (Mts. Daisetsu, Mt. Shirouma, Mts. Yatsugadake and Mts. Arakawa). They are recommended to be revised taxonomically.

Each cpDNA haplotype in a few species was geographically structured: *Solidago virga-aurea*, *Pedicularis yezoensis*, and *Aquilegia flabellata* Siebold et Zucc. var. *pumila* (Huth) Kudô (Ranunculaceae). In *P. yezoensis*, for example, five haplotypes were observed (Type A-E), and the haplotypes of the populations of Hokkaido (Type A, Mt. Yotei; Type B, Mts. Poroshiri) in Japan, and northern Honshu (Type C, Mt. Gassan; Type D, Mts. Iide) possessed a common nucleotide substitution, respectively.

On the other hand, the geographic structure of cpDNA haplotypes was not recognized in several other taxa: alpine species of *Taraxacum* and *Anaphalis margaritacea* (L.) Benth. et Hook. f. (Asteraceae), *Veronica schmidtiana*, *Peucedanum multivittatum* Maxim. (Umbelliferae) and *Trollius riederianus*. In *V. schmidtiana*, for instance, the haplotype of the population of Mt. Rausu in Hokkaido (Type A) also observed in that of Mt. Shirouma in Honshu, although the other populations in Hokkaido possessed another haplotypes (Type B, Rishiri Island; Type C, Mt. Yubari).

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Table 1. Intraspecific variation of cpDNA in Japanese alpine plants

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
<b>Asteraceae</b>		
<b>Japanese alpine <i>Taraxacum</i> F. H. Wigg.</b>	330-407 bp	
<i>T. trigonolobum</i> Dahlst.		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F154
Mt. Yotei, Hokkaido, Japan, NF	B (NS, 2 bp; 1 Indel, 77 bp)	F522
<i>T. alpicola</i> Kitam.		
var. <i>shiroumense</i> (H. Koidz.) Kitam.		
Mt. Shirouma, Nagano, Honshu, Japan, NS	C (NS, 3 bp; 2 Indels, 31, 115 bp)	F788
<i>T. yatsugatakense</i> H. Koidz.		
Mt. Kitadake, Yamanashi, Honshu, Japan, NS	B	F915
<b>Japanese <i>Leontopodium</i> R. Br. ex Cass.</b>	411 bp	
<i>L. discolor</i> P. Beauv.		
Rebun Island, Hokkaido, Japan, NF	A	F63
<i>L. hayachinense</i> (Takeda) H. Hara et Kitam.		
Mt. Hayachine, Iwate, Honshu, Japan, NF	A	F682
<b><i>Anaphalis margaritacea</i> (L.) Benth. et Hook. f.</b>	386-396 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S678
Mt. Nipesotsu, Hokkaido, Japan, NF	A	S1148, 1151
Mt. Rausu, Hokkaido, Japan, NF	B (NS, 1 bp)	S655
Mt. Yubari, Hokkaido, Japan, NF	A	S924
Mt. Yotei, Hokkaido, Japan, NF	A	S971
Mt. Apoi, Hokkaido, Japan, NF	A	S904
Rishiri Island, Hokkaido, Japan, NF	A	S716
Rebun Island, Hokkaido, Japan, NF	A	S719
Mts. Hakkoda, Aomori, Honshu, Japan, NF	C (1 Indel, 10 bp)	S964
Mt. Iwaki, Aomori, Honshu, Japan, NS	A	S873
Mt. Hayachine, Iwate, Honshu, Japan, NF	A	S1097
Mt. Akitakomagadake, Akita, Honshu, Japan, NF	D (NS, 2 bp)	S961
Mts. Iide (Mt. Mikuni), Fukushima, Honshu, Japan, NF	A	S1107
Mts. Iide (Mt. Eboshi), Yamagata, Honshu, Japan, NF	D	S1256
Mt. Amakazari, Nagano, Honshu, Japan, NS	B	S1208
Mt. Ariake, Nagano, Honshu, Japan, TS	B	S885
Yari-Spa, Hakuba, Nagano, Honshu, Japan, TS	D	S1207
Mts. Hakusan (Mt. Nanakura), Ishikawa, Honshu, Japan, TS	B	S67
Mts. Hakusan (Mikaeri-zaka), Ishikawa, Honshu, Japan, TS	B	S118
Mt. Ontake, Nagano, Honshu, Japan, TS	D	S1211
Mt. Kisokomagadake, Nagano, Honshu, Japan, NS	B	S1210
Mts. Yatsugadake, Nagano, Honshu, Japan, NS	B	S1217
Mt. Kitadake, Yamanashi, Honshu, Japan, NS	B	S1214
Todai, Hase, Nagano, Honshu, Japan, NS	A	S1252
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	B	S1212
Mt. Akaishi, Shizuoka, Honshu, Japan, NS	B	S1213
Sakhalin, T. Fukushima	A	S1013
Mt. Pulchoki, Nepal, TS	A	S1268
<b><i>Solidago virga-aurea</i> L.</b>		
( <i>trnL-trnF</i> )	393-398 bp	
Mts. Daisetsu (Ginsendai), Hokkaido, Japan, NF	A	S361, 362
Mts. Daisetsu (Hakuun), Hokkaido, Japan, NF	A	S366, 367
Mt. Kamihorokamettoku, Hokkaido, Japan, T. Sato	A	S407, 408
Mt. Moiwa, Sapporo, Hokkaido, Japan, NS	A	S441, 443
Nakayama Pass, Sapporo, Hokkaido, Japan, KO	A	S402
Mt. Sapporodake, Hokkaido, Japan, NS	A	S410
Mt. Teinesan, Sapporo, Hokkaido, Japan, T. Sato	A	S436
Mt. Apoi, Hokkaido, Japan, T. Sato	B (1 Indel, 5 bp)	S447
Mts. Iide (Mt. Tanemaki), Fukushima, Honshu, Japan, TS	B	S264
Mts. Iide (Mt. Mikuni), Fukushima, Honshu, Japan, TS	B	S266
Mts. Iide (Mt. Mikuni), Fukushima, Honshu, Japan, TS	C (NS, 1 bp; 1 Indel, 5 bp)	S265
Mts. Hakusan (Ohnanjimime), Ishikawa, Honshu, Japan, TS	B	S12
Mts. Hakusan (Kozakura-daira), Ishikawa, Honshu, Japan, TS	B	S26, 27

Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
Mts. Hakusan (Bettou-Nozoki), Ishikawa, Honshu, Japan, TS	B	S1, 2
Mt. Ontake, Nagano, Honshu, Japan, TS	B	S181, 182
Tien Shan Range, China, TS	B	S529
Austria (from KBG <sup>4</sup> ), TS	B	S485, 486, 487
Scotland (from EBG <sup>4</sup> ), TS	B	S468
<i>(trnL intron)</i>	about 450 bp	
Mts. Daisetsu (Ginsendai), Hokkaido, Japan, NF	A	S361
Mts. Daisetsu (Hakuun), Hokkaido, Japan, NF	B (1 Indel, 5 bp)	S366
Mt. Kamihorokamettoku, Hokkaido, Japan, T. Sato	A	S406
Mt. Moiwa, Sapporo, Hokkaido, Japan, NS	A	S441
Nakayama Pass, Sapporo, Hokkaido, Japan, KO	A	S401
Mt. Sapporodake, Hokkaido, Japan, NS	A	S410
Mt. Teinesan, Sapporo, Hokkaido, Japan, T. Sato	A	S436
Mt. Apoi, Hokkaido, Japan, T. Sato	A	S447
Mts. Iide (Mt. Tanemaki), Fukushima, Honshu, Japan, TS	A	S264
Mts. Iide (Mt. Mikuni), Fukushima, Honshu, Japan, TS	A	S266
Mts. Iide (Mt. Mikuni), Fukushima, Honshu, Japan, TS	A	S265
Mts. Hakusan (Ohnanjimine), Ishikawa, Honshu, Japan, TS	A	S11
Mts. Hakusan (Kozakura-daira), Ishikawa, Honshu, Japan, TS	A	S26
Mts. Hakusan (Bettou-Nozoki), Ishikawa, Honshu, Japan, TS	A	S1
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S181,
Tien Shan Range, China, TS	A	S529
Austria (from KBG <sup>4</sup> ), TS	A	S485, 486
Scotland (from EBG <sup>4</sup> ), TS	A	S468
<i>Artemisia arctica</i> Less.	396 bp	
var. <i>villosa</i> (Koidz.) Tatew. ex Kitam.		
Rishiri Island, Hokkaido, Japan, NF	A	F4
var. <i>sachalinensis</i> F. Schmidt		
Mt. Hayachine, Iwate, Honshu, Japan, NF	A	F666
<b>Campanulaceae</b>		
<i>Campanula lasiocarpa</i> Cham.	367 bp	
Unalaska Island, the Aleutian Islands, U. S. A., YW	A	FCL14
Mt. Rausu, Hokkaido, Japan, NF	A	FCL7
Mts. Daisetsu, Hokkaido, Japan, NF	A	FCL3
Mts. Poroshiri, Hokkaido, Japan, NF	A	FCL5
Mt. Iwate, Iwate, Honshu, Japan, NF	A	FCL9
Mts. Hakusan, Ishikawa, Honshu, Japan, NS	B (NS, 1 bp)	FCL11
<i>Campanula chamissonis</i> Fed.	396 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F212
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F871
<b>Rubiaceae</b>		
<i>Galium kamtschaticum</i> Steller ex Roem. et Schltld.	395 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S302
Mts. Hakusan (Kuroyuri-dani), Ishikawa, Honshu, Japan, NS	A	S161
Mts. Hakusan (Mikaeri-zaka), Ishikawa, Honshu, Japan, NS	A	S171
Mts. Hakusan (Mt. Nanakura), Ishikawa, Honshu, Japan, NS	B (NS, 1 bp)	S76
<b>Scrophulariaceae</b>		
<i>Pennellianthus frutescens</i> (Lamb.) Crosswh.	339 bp	
Mt. Rausu, Hokkaido, Japan, NF	A	F114
Mt. Akitakomagadake, Akita, Honshu, Japan, NF	A	F596
<i>Veronica schmidtiana</i> Regel ex F. Schmidt	354 bp	
var. <i>schmidtiana</i>		
Mt. Rausu, Hokkaido, Japan, NF	A	F101
Rishiri Island, Hokkaido, Japan, NF	B (NS, 1 bp)	F44

Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
var. <i>yezo-alpina</i> (Koidz.) T. Yamaz. Mt. Yubari, Hokkaido, Japan, NF	C (NS, 2 bp)	F333
var. <i>senanensis</i> (Maxim.) Ohwi Mt. Shirouma, Nagano, Honshu, Japan, NS	A	F796
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	D (NS, 3 bp)	F858
<b><i>Veronica stelleri</i> and <i>V. nipponica</i></b>	392bp	
<i>V. stelleri</i> Pall. ex Link var. <i>longistyla</i> Kitag. Mts. Daisetsu, Hokkaido, Japan, NF	A	F168
<i>V. nipponica</i> Makino ex Matsum. var. <i>shinano-alpina</i> H. Hara Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F884
<b><i>Pedicularis yezoensis</i> Maxim.</b>	374-380 bp	
Mts. Poroshiri, Hokkaido, Japan, NF	A	F476
Mt. Yotei, Hokkaido, Japan, NF	B (NS, 1 bp)	F524
Mt. Gassan, Yamagata, Honshu, Japan, NF	C (NS, 3 bp)	F2022
Mts. Iide, Yamagata, Honshu, Japan, NF	D (NS, 4 bp)	F746
Mts. Hakusan, Ishikawa, Honshu, Japan, TS	E (NS, 2 bp; 1 Indel, 6 bp)	F1103
<b><i>Pedicularis verticillata</i> L.</b>	378-380bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F151
Mt. Shirouma, Nagano, Honshu, Japan, NS	B (2 Indels, 2 and 1 bp)	F810
Mts. Hakusan, Ishikawa, Honshu, Japan, NS	C (NS, 8 bp; 3 Indels, 2, 2 and 1 bp)	F1165
Mts. Yatsugadake, Nagano, Honshu, Japan, NS	D (3 Indels, 2, 1 and 1 bp)	F943
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	D	F893
<b><i>Pedicularis apodochila</i> Maxim.</b>	432bp	
Mt. Tottabetsu, Hokkaido, Japan, NF	A	F444
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F877
<b>Gentianaceae</b>		
<b><i>Gentiana nipponica</i> Maxim.</b>	312-341bp	
var. <i>nipponica</i> Mts. Daisetsu, Hokkaido, Japan, NF	A	F163
Mt. Gassan, Yamagata, Honshu, Japan, NF	B (NS, 1 bp; 1 Indel, 14 bp)	FGA31
Mts. Iide, Fukushima, Honshu, Japan, NF	C (NS, 1 bp)	F726
Mt. Kisokomagadake, Nagano, Honshu, Japan, NS	D (NS, 3 bp; 1 Indel, 7 bp)	F843
var. <i>robusta</i> H. Hara Mts. Iide, Fukushima, Honshu, Japan, NF	E (NS, 3 bp; 1 Indel, 15 bp)	F729
<b><i>Gentiana algida</i> Pall.</b>	340-350bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	FGA8
Mt. Gassan, Yamagata, Honshu, Japan, NF	B (NS, 1 bp; 1 Indel, 7 bp)	FGA2
Mt. Shirouma, Nagano, Honshu, Japan, NF	B	FGA20
Mt. Noguchigoro, Nagano, Honshu, Japan, NF	B	FGA17
Mts. Hakusan, Ishikawa, Honshu, Japan, NS	B	FGA1
Mt. Kisokomagadake, Nagano, Honshu, Japan, Y. Kita	C (NS, 3 bp; 1 Indel, 8 bp)	FGA23
Mts. Arakawa, Shizuoka, Honshu, Japan, TS	D (NS, 1 bp; 1 Indel, 2 bp)	FGA34
Baikal Lake, Russia, M. Todoroki	E (NS, 1 bp; 1 Indel, 2 bp)	FGA14
Altai Range, Mongol, M. Amano	F (NS, 3 bp; 1 Indel, 2 bp)	FGA13
<b>Ericaceae</b>		
<b><i>Vaccinium vitis-idaea</i> L.</b> ( <i>trnL-trnF</i> )	410bp	
Mts. Daisetsu, Hokkaido, Japan, TK and KO	A	S276
Mt. Esan, Esan, Hokkaido, Japan, T. Sato	A	S465
Mts. Hakkoda, Aomori, Honshu, Japan, MI	A	S466
Mt. Kurikoma, Miyagi, Honshu, Japan, MI	A	S450
Mt. Shirouma, Nagano, Honshu, Japan, NF	A	S431

Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
Mt. Noguchigoro, Nagano, Honshu, Japan, TS	B (NS, 1 bp)	S460
Mt. Mitsugadake, Nagano, Honshu, Japan, TS	A	S458
Mt. Takabocchi, Okaya, Nagano, Honshu, Japan, TS	C (NS, 1 bp)	S42
Mt. Hachibuse, Okaya, Nagano, Honshu, Japan, TS	C	S46
Mts. Hakusan, Ishikawa, Honshu, Japan, TS	A	S86
Mt. Ontake, Nagano, Honshu, Japan, TS	D (NS, 1 bp)	S191
Nizke-Tatry Range, Slovakia, TS	A	S577
Baikal Lake, Russia, M. Todoroki	A	S448
<i>(trnL intron)</i>	490 bp	
Mts. Hakkoda, Aomori, Honshu, Japan, MI	A	S457
Mt. Shirouma, Nagano, Honshu, Japan, NF	A	S431
Mt. Mitsugadake, Nagano, Honshu, Japan, TS	A	S458
Mts. Hakusan (Mikaeri-zaka), Ishikawa, Honshu, Japan, TS	A	S111
Mts. Hakusan (Ohnanjimimine), Ishikawa, Honshu, Japan, TS	A	S86
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S191
<b>Cornaceae</b>		
<i>Chamaepericlymenum canadense</i> (L.) Asch. et Graebn.		
	374 bp	
Mts. Daisetsu, Hokkaido, Japan, TK and KO	A	S308
Mts. Hakusan (Ohnanjimimine), Ishikawa, Honshu, Japan, TS	A	S62
Mts. Hakusan (Kozakura-daira), Ishikawa, Honshu, Japan, TS	A	S136
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S211
<b>Umbelliferae</b>		
Japanese <i>Coelopleurum</i> Ledeb.		
	373 bp	
<i>C. trichocarpum</i> (H. Hara) Kitag.		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F174
<i>C. multisectum</i> (Maxim.) Kitag.		
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F895
<i>Peucedanum multivittatum</i> Maxim.		
	407-408 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F160
Mt. Hayachine, Iwate, Honshu, Japan, NF	B (NS, 1 bp)	F114-6
Mts. Iide, Fukushima, Honshu, Japan, NF	C (1 Indel, 1 bp)	F731
Mts. Hakusan, Ishikawa, Honshu, Japan, TS	B	F111.4
<b>Onagraceae</b>		
<i>Epilobium angustifolium</i> L.		
<i>(trnL-trnF)</i>	251-295 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S286
Mts. Daisetsu, Hokkaido, Japan, NF	B (1 Indel, 44 bp)	S287
Kusharo Lake, Hokkaido, Japan, TK and KO	A	S413, 437
Mt. Moiwa, Sapporo, Hokkaido, Japan, NS	B	S444, 445
Mt. Noguchigoro, Nagano, Honshu, Japan, TS	A	S414
Yunomata Spa, Ohmachi, Nagano, Honshu, Japan, TS	A	S440
Mt. Hachibuse, Okaya, Nagano, Honshu, Japan, TS	A	S419, 420
Mt. Takabocchi, Okaya, Nagano, Honshu, Japan, TS	A	S421
Mt. Norikura, Nagano, Honshu, Japan, TS	A	S432, 435
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S236, 237
Mts. Arakawa, Shizuoka, Honshu, Japan, FK	A	S569
Tien Shan Range, China, TS	C (1 Indel, 11 bp; NS, 2 bp)	S546
Tien Shan Range, China, TS	A	S553
Nizke-Tatry Range, Slovakia, TS	D (1 Indel, 44 bp; NS, 1 bp)	S534
<i>(trnL intron)</i>	about 550 bp	
Kusharo Lake, Hokkaido, Japan, TK and KO	A	S413
Mt. Hachibuse, Okaya, Nagano, Honshu, Japan, TS	A	S419
Yunomata Spa, Ohmachi, Nagano, Honshu, Japan, TS	A	S440

Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
Mt. Norikura, Nagano, Honshu, Japan, TS	A	S435
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S236
Mts. Arakawa, Shizuoka, Honshu, Japan, FK	A	S569
Nizke-Tatry Range, Slovakia, TS	A	S534
Tien Shan Range, China, TS	B (1 Indel, 9 bp)	S546
<b>Violaceae</b>		
<i>Viola biflora</i> L.		
	376 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S386
Mt. Shirouma, Nagano, Honshu, Japan, TS	A	S483
Mts. Hakusan (Murodo), Ishikawa, Honshu, Japan, NF	A	S297
Mts. Hakusan (Mt. Nanakura), Ishikawa, Honshu, Japan, NF	A	S106
Mts. Hakusan (Kuroboko-iwa), Ishikawa, Honshu, Japan, NF	A	S246
Yunnan, China (from EBG <sup>4</sup> ), TS	B (NS, 2 bp)	S484
Tien Shan Range, China, TS	C (NS, 3 bp)	S469
<i>Viola crassa</i> Makino		
	340 bp	
var. <i>borealis</i> (Hid. Takah.) T. Shimizu		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F2181
var. <i>crassa</i>		
Mt. Iwate, Iwate, Honshu, Japan, NF	A	F2182
<b>Empetraceae</b>		
<i>Empetrum nigrum</i> L. var. <i>japonicum</i> K. Koch		
	411-412 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S281
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S201
Mts. Hakusan, Ishikawa, Honshu, Japan, NF	B (1 Indel, 1 bp)	S256
Alberta, U. S. A. (from KBG <sup>4</sup> ), TS	C (1 Indel, 1 bp)	S492, 609
<b>Oxalidaceae</b>		
<i>Oxalis acetosella</i> L.		
	378 bp	
Mts. Hakusan, Ishikawa, Honshu, Japan, NF	A	S129
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S231
<b>Leguminosae</b>		
<i>Oxytropis nigrescens</i> Fisch.		
	158 bp	
var. <i>sericea</i> (Koidz.) T. Shimizu		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F229
var. <i>japonica</i> (Maxim.) Hultén		
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F889
<i>Hedysarum vicioides</i> Turcz.		
	267 bp	
Mt. Yubari, Hokkaido, Japan, NF	A	F390
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	A	F882
<b>Rosaceae</b>		
<i>Potentilla matsumurae</i> Th. Wolf		
	430 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F185
Mt. Akaishi, Shizuoka, Honshu, Japan, NS	A	F872
<i>Geum calthifolium</i> Sm.		
var. <i>nipponicum</i> (F. Bolle) Ohwi	449 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F227
Mts. Hakusan, Ishikawa, Honshu, Japan, NF	A	F1145
<b>Saxifragaceae</b>		
<i>Saxifraga fusca</i> Maxim.		
	368 bp	
var. <i>kurilensis</i> Ohwi		
Mt. Shari, Hokkaido, Japan, NF	A	F143
var. <i>fusca</i>		
Mt. Yubari, Hokkaido, Japan, NF	A	F326



Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
Mt. Hayachine, Iwate, Honshu, Japan, NF	B (NS, 2 bp)	F1149
var. <i>kikubuki</i> Ohwi		
Mts. Iide, Fukushima, Honshu, Japan, NF	A	F708
Mts. Hakusan, Ishikawa, Honshu, Japan, NF	C (NS, 1 bp)	F1143
<b>Crassulaceae</b>		
<i>Sedum rosea</i> (L.) Scop.	319 bp	
Rebun Island, Hokkaido, Japan, NF	A	F84
Mts. Hakusan, Ishikawa, Honshu, Japan, NF	A	F1139
<i>Sedum ishidae</i> Miyabe et Kudô	315 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	F216
Mts. Iide, Niigata, Honshu, Japan, NF	A	F747
<b>Ranunculaceae</b>		
<i>Ranunculus acris</i> L. var. <i>nipponicus</i> H. Hara	407 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S291
Mts. Hakusan, Ishikawa, Honshu, Japan, TS	A	S56
<i>Trautvetteria caroliniensis</i> (Walter) Vail	423-428 bp	
Mts. Daisetsu, Hokkaido, Japan, NF	A	S381
Mts. Hakusan, Ishikawa, Honshu, Japan, TS	B (1 Indel, 5 bp)	S71
Mt. Ontake, Nagano, Honshu, Japan, TS	A	S221
<i>Trollius riederianus</i> Fisch. et C. A. Mey.	420-421 bp	
var. <i>riederianus</i>		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F215
var. <i>pulcher</i> (Makino) T. Shimizu		
Rishiri Island, Hokkaido, Japan, NF	B (1 Indel, 1 bp)	F33
var. <i>japonicus</i> (Miq.) Ohwi		
Mt. Shari, Hokkaido, Japan, NF	A	F123
Mts. Hakkoda, Aomori, Honshu, Japan, NF	A	F569
Mt. Shirouma, Nagano, Honshu, Japan, NS	C (NS, 3 bp; 1 Indel, 1 bp)	F760
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	D (NS, 2 bp; 1 Indel, 1 bp)	F899
<i>Aquilegia flabellata</i> Siebold et Zucc.		
var. <i>pumila</i> (Huth) Kudô	about 450 bp	
Rebun Island, Hokkaido, Japan, NF	A	F78
Mt. Shari, Hokkaido, Japan, NF	A	F147
Mt. Hayachine, Iwate, Honshu, Japan, NF	B (1 Indel, 4 bp)	F660
Mt. Shirouma, Nagano, Honshu, Japan, NS	B	F764
Mts. Arakawa, Shizuoka, Honshu, Japan, NS	B	F900
<b>Caryophyllaceae</b>		
<i>Arenaria arctica</i> Steven ex Ser.	406 bp	
var. <i>rebunensis</i> T. Shimizu		
Rebun Island, Hokkaido, Japan, NF	A	F53
var. <i>arctica</i>		
Mts. Daisetsu, Hokkaido, Japan, NF	A	F230
var. <i>hondoensis</i> (Ohwi) H. Hara		
Mt. Akaishi, Shizuoka, Honshu, Japan, NS	B (NS, 1 bp)	F866
<i>Stellaria nipponica</i> Ohwi	407 bp	
Mt. Yubari, Hokkaido, Japan, NF	A	F321
Mt. Kitadake, Yamanashi, Honshu, Japan, NS	A	F908
<b>Orchidaceae</b>		
<i>Orchis aristata</i> Fisch.	291 bp	
Unalaska Island, the Aleutian Islands, U. S. A., YW	A	FOA58
Akutan Island, the Aleutian Islands, U. S. A., YW	A	FOA59

Table 1. Continued

Taxon, Locality and Collector <sup>1</sup>	Length and Haplotype <sup>2</sup>	Sample No. <sup>3</sup>
Rishiri Island, Hokkaido, Japan, NF	A	FOA1
Mt. Rausu, Hokkaido, Japan, NF	A	FOA17
Mts. Daisetsu, Hokkaido, Japan, NF	A	FOA6
Mts. Poroshiri, Hokkaido, Japan, NF	A	FOA22
Mt. Iwaki, Aomori, Honshu, Japan, NF	A	FOA27
Mt. Iwate, Iwate, Honshu, Japan, NF	A	FOA32
Mt. Chokai, Yamagata, Honshu, Japan, M. Yamashita	A	FOA56
Mt. Gassan, Yamagata, Honshu, Japan, NF	A	FOA37
Mts. Iide, Fukushima, Honshu, Japan, S. Shindo	A	FOA42
Mts. Hakusan, Ishikawa, Honshu, Japan, NS	A	FOA52
Mt. Kitadake, Ishikawa, Honshu, Japan, T. Abe	A	FOA47
<b>Liliaceae</b>		
<i>Fritillaria camtschaticensis</i> (L.) Ker Gawl.	198-199 bp	
Unalaska Island, the Aleutian Islands, U. S. A., YW	A	FFC48
Akutan Island, the Aleutian Islands, U. S. A., YW	A	FFC49
Mts. Daisetsu, Hokkaido, Japan, NF	B (1 Indel, 1 bp)	FFC4
Sarufutsu River, Hokkaido, Japan, KO (Triploidy)	B	FFC1
Mts. Hakusan, Ishikawa, Honshu, Japan, NS	B	FFC37
Mt. Kitadake, Yamanashi, Honshu, Japan, T. Abe	B	FFC14

1 NF, N. Fujii; NS, N. Shirai; TS, T. Shimizu; KO, K. Ohsawa; YW, Y. Watanabe; TK, T. Kitabatake; MI, M. Imazu; FK, F. Konta.

2 The polymorphic characters against Haplotype are shown in parentheses: NS, nucleotide substitution; Indel, insertion/deletion.

3 Bold numbers represent the sequenced samples in both directions.

4 KBG, Royal Botanic Gardens, Kew; EBG, Royal Botanic Gardens, Edinburgh.

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### 摘要

著者らはこれまでにエゾコザクラ *Primula cu-*

*neifolia* (サクラソウ科) とヨツバシオガマ *Pedicularis chamissonis* (ゴマノハグサ科) の2種において葉緑体 DNA の種内変異を報告してきた (Fujii *et al.* 1995, Fujii *et al.* 投稿中)。両種は、日本では亜高山帯から高山帯にかけて生育する高山植物であり、幾つかの種内分類群が認められている。それらの研究によると、各々の種内において多くの葉緑体 DNA タイプが認められ、さらにそのタイプ間の系統関係を推定することが可能であった。

他の高山植物においてどのような遺伝的分化が見られるかは興味深いところである。そこで本研究では、日本産高山植物から40分類群(36種と4種群)を材料として、葉緑体 DNA の種内変異及び種間変異の検出を試みた。これら40分類群は必ずしもエゾコザクラやヨツバシオガマのような外部形態的に識別できる種内分類群などが知られているものに限ったわけではなく、ある程度広範な分布域を有するものをランダムにサンプリングしたものである。上記2種において有効な情報を示した葉緑体 DNA の *trnL* (UAA) 3'exon~*trnF* (GAA) の遺伝子間領域と、一部の分類群については *trnL* (UAA) のイントロンの塩基配列を決定し、その変異を解析した。

その結果、表1に見られるように、19分類群では葉緑体 DNA の変異はまったく検出されなかった。例えば、ハクサンチドリ *Orchis aristata* (ラン科) では南アルプスの北岳からアリューシャン列島のウナラスカ高にかけて13集団を調べた結果まったく変異がなかった。しかし解析した集団数の少ない分類群がほとんどなので、19分類群すべてにおいて多型がないとは言明しがたい。

変異の見られた21分類群のうち8分類群において、系統学的な知見や地域的なまとまりを示唆する

ような多型が得られた。その中で高山生タンポポ属 *Taraxacum* (キク科)、ミヤマクワガタ *Veronica schmidtiana*, エゾシオガマ *Pedicularis yezoensis*, タカネシオガマ *P. verticillata* (ゴマノハグサ科)、ミヤマリンドウ *Gentiana nipponica* (リンドウ科)、シナノキンバイ *Trollius riederianus* (キンボウゲ科) ではかなりの多型が検出された。たとえば高山生タンポポ群やミヤマクワガタ、ミヤマリンドウでは、挿入/欠失を含めた多型サイトの割合がエゾコザクラやヨツバシオガマのそれと同程度であった。またエゾシオガマでは、多型サイトの割合は多少小さいにもかかわらず、北海道(羊蹄山と幌尻岳)と本州(月山と飯豊山)の集団の葉緑体 DNA タイプをそれぞれにまとめる塩基置換が検出され、地域的なまとまりが示唆された。

タカネシオガマでは興味深い知見が得られた。白山のタカネシオガマは他山系のものに比べて、花序が長く伸びて全体としてよりスマートな形をしており、外部形態的にかなり分化しているものと思われる。今回の葉緑体 DNA のデータからも、白山のタカネシオガマは遺伝的にもかなり分化していることが明らかにされた(大雪山のタカネシオガマに対して8bpの塩基置換と3つの挿入/欠失を持っていた)。今後、白山のタカネシオガマの分類学的再検討が必要であろう。

以上のように、今回解析した分類群の大半は種内変異及び種間変異が示されなかったが、一部の分類群ではエゾコザクラやヨツバシオガマで見られたような遺伝的分化が明らかとなった。このようなデータの蓄積は、日本産高山植物の遺伝的分化に関する基礎資料として重要であると思われる。

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