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IV. Peroxidase Isozyme Variation in Solidago virgaurea Complex along an Altitudinal Gradient on Mt. Norikura, Central Japan

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Summary

By using *Solidago Virgaurea* Complex which widely distributed from basal to alpine zones, some morphological and ecological characteristics and peroxidase isozyme variation were investigated along an altitudinal gradient.

In the case of the mature plants in the field, some altitudinal differences in morphological and ecological characteristics were clear between zones below and above 2000 or 2200m alt., and the number of main peroxidase isozymes was increased till 2200m alt. with the increase of their habitat altitude, but decreased at 2370m alt.. In this progress, the appearances of some isozymes were changed among altitudinal different plant populations such as absence, rare presence (minors), or mostly presence (mains) in the plants. When the seedlings which were derived from the seeds collected at different altitudes were grown under controlled conditions, the mobility of main isozymes was larger in the seedlings from high altitudes than in these from low ones.

The variation in the number and the mobility of main isozymes with the increase of habitat altitude seems to correspond to altitudinal speciation from *S. Virgaurea* ssp. *asiatica* to ssp. *leiocarpa*.

Introduction

Since TURESSON[19] proposed a definition of ecotype, many workers[e.g.,2,3,6,9,12,13,17,18,20] have been interested in the differences in some physiological or ecological characteristics of plants, which are in the same species, growing in various environmental conditions, and these differences have been investigated in relation to intraspecific variation or speciation. *Solidago Virgaurea* complex, widely distributed in various environmental conditions, has often been used for this kind of study[1,2,9,18,20]. It is difficult, however, to conclude genetic differentiation from the ecological characteristics alone when a morphological change in the species is continuous or hardly shown along an environmental gradient. In this case, isozyme analysis is considered to be useful for the systematic study of plants because isozymes have a close relation with their genes.

On the other hand, it has been reported that the activity of isozymes in some enzymes is changed by the environmental conditions in which the plants grew[4,7,10,11,15,16,22]. On cytochrome oxidase isozymes, it has been reported that an isozyme composition in *Polygonum cuspidatum* seedlings differed from that in its mature plants, and that some of the isozymes were detected only when plants were grown at a relatively low temperature[22]. Therefore, special care would be required for a genetic analysis by using isozymes in plants in the field.

This paper will report peroxidase isozyme variation in *S. Virgaurea* complex with a change of some morphological and ecological characters along an altitudinal gradient.

Materials and Methods

Mature plants of *Solidago Virgaurea* complex growing in sunny places at 700, 1500, 2000, 2200, and 2370m alt. along the eastern slope of Mt. Norikura, central Japan, were used to research morphological and ecological characteristics. The number of clones per plant, the presence or absence of radical leaves on bolting plant, flowering season, and the morphology of involcural scales were investigated in each altitudinal habitat.

Peroxidase isozymes were analyzed on the mature leaves of flowering *S. Virgaurea* complex collected at 30m alt. (Kaga, Kanazawa Prefecture) on the Japan Sea side and at 700, 2000, 2200, and 2370m alt. along the eastern slope of Mt. Norikura. The isozyme analysis for its seedlings was taken on these grown for about 2 weeks at 20°C under a continuons light of 24 hr. after the seeds collected at 700 and 2370m alt. were germinated at 20°C. On the seedlings, a whole plant was used for analysis. The collection site on the Japan Sea side located at about 120 km away to the west of Mt. Norikura was in about the same latitude to the mountain, but was geographically separated by high mountains of about 3000m alt..

The mature leaves collected in the field were carried to the laboratory with ice-cooling. On arrival, 0.5 g fresh leaves for the mature plants or the whole plant for seedlings were homogenized with 5 ml of 1/15 M phosphate buffer (pH 7.0), and the homogenates were stocked at -20°C until analysis. After thawing for analysis, the homogenates were centrifuged for 45 min. with 3000 r.p.m.. These supernatants were applied to polyacrylamide gel, pH 8.3, and electrophoresis was run for 4 hr. with 200 V. All of these procedures for analysis were taken at 4°C. Peroxidase isozymes were analyzed on 6 plants at 30m alt., 18 at 700m, 23 at 2000m, 26 at 2200m, and 40 at 2370m, and on 30 seedlings derived from 700m and 2370m alt., respectively.

After electrophoresis, the gel was stained for peroxidase by the method of YAMAMOTO and MOMOTANI[21]. It has been reported on *Polygonum cuspidatum* that some isozymes in cytochrome oxidase were specifically stained at a low temperature[22]. A similar specific staining on peroxidase isozymes was preliminarily tested at 4°, 25°, and 32°C, but all of peroxidase isozymes in *S. Virgaurea* complex were never found to show such specific staining. Thereafter, staining was taken at 20°C. Stained peroxidase isozymes were compared to each other by a relative mobility (pI value).

Results

Morphological and ecological characteristics on *S. Virgaurea* complex were shown in Table 1 and Fig. 1. With the increase of these habitat altitude, the number of clones per plant was increased, and radical leaves had a tendency to remain after bolting. The flowering season was hastened with the increase of the habitat altitude, and its difference was clear between habitats below 1500m and above 2000m alt.. Morphological difference in involucural scales, also, was found between 1500m and 2000m alt..

Table 1 Ecological and morphological characteristics of *Solidago virgaurea* complex at various altitudes on Mt. Norikura.

Characteristics \ Altitude (m)	700	1500	2000	2200	2370
Number of clones per plant	a few	a few	a few	a few	much
Radical leaves on bolting plants	no	no	present on some plants	present on some plants	present
Flowering season	Sep.-Oct.	Sep.	Jul.-Aug.	Jul.-Aug.	Jul.-Aug.

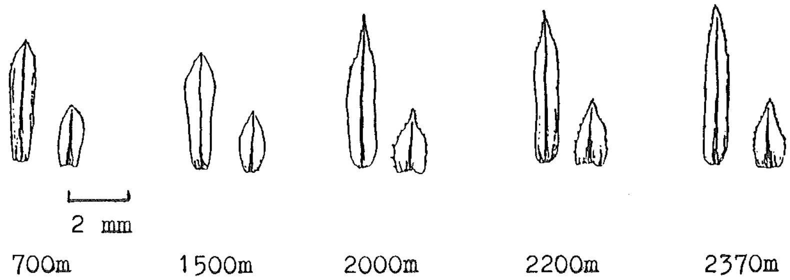

Fig. 1 Morphological characteristics of involucural scales of *Solidago virgaurea* complex at various altitudes on Mt. Norikura. Left : inners, right : outers.

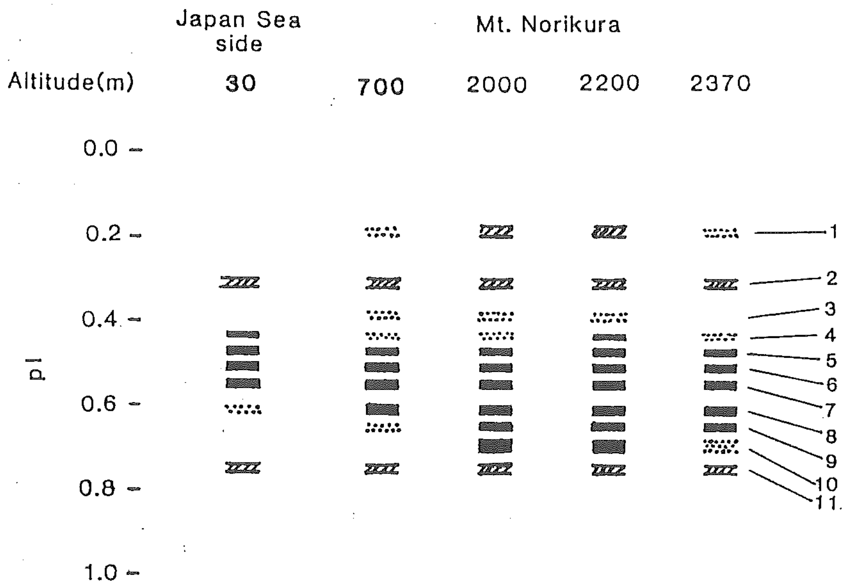

Fig. 2 Peroxidase isozymes in the mature plants of *Solidago virgaurea* complex collected from different altitudes. Solid and dote lines show main and minor isozymes, respectively, and darkened bands in the main isozymes mean a deeper staining than obliques.

The number of peroxidase isozymes varied a little among individual plants in a population at the same altitude of habitat, and clearly differed among plant populations at different altitudes of habitat. To such variation on the number of the isozymes, when an isozyme showing the same mobility was found in plants numbering over 30% in a population, the isozyme was called the main isozyme, and an isozyme with a lower frequency than 30% was called a minor one. All the isozymes found at each habitat altitude were shown in Fig. 2. The isozymes in the mature plants were detected to be 11 elements showing different pI value to each other, and the plants in a population had all or some of these isozymes. These 11 isozymes were numbered from an isozyme having the lowest pI value to that having the highest one.

The total number of the isozymes was the most (11 elements) at 2000m and 2200m alt., and was the fewest (7 elements) at 30m alt.. The number of main isozymes in the mature plants on Mt. Norikura varied from 6 at 700m alt. to 10 at 2200m. Although the main isozymes at 30m alt. on the Japan Sea side were the same number as those at 700m alt. on Mt. Norikura, some of those at 700m alt. had a tendency to appear at a higher value than at 30m, and the total number of the isozymes was much more at 700m alt. than at 30m alt..

With the increase of the habitat altitude on the eastern slope of Mt. Norikura, a

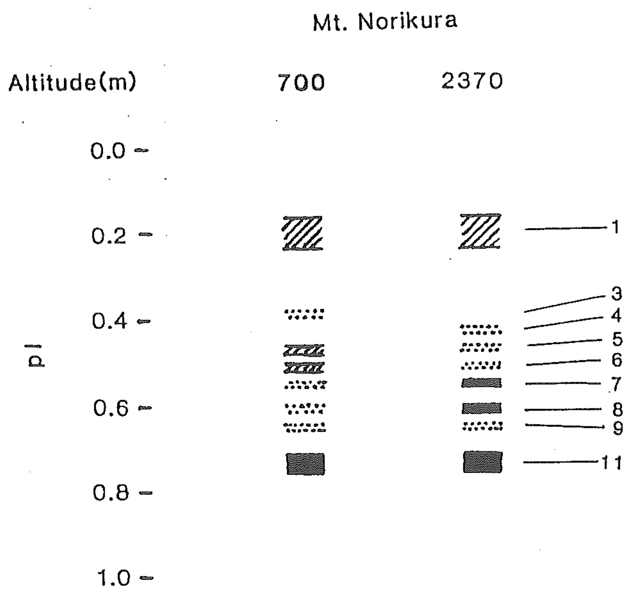

Fig. 3 Peroxidase isozymes in the seedlings of *Solidago virgaurea* complex derived from its seeds at 700 m and 2370 m altitudes. Solid and dote lines show main and minor isozymes, respectively, and darkened bands in the main isozymes mean a deeper staining than obliques.

variation of the isozymes in the mature plants was characterized by a change from minor isozymes to main ones in some isozymes or by the appearance of the new isozyme, and then, by a change from main isozymes to minor ones or the disappearance of an isozyme at 2370m alt.. A similar change in the isozymes was found between the plants at 30m alt. on the Japan Sea side and at 700m alt. on Mt. Norikura.

Each isozyme in the seedlings was identical to some of the isozymes in the mature plants on its mobility. For that reason, the identical isozyme was numbered with the same number to that in the mature plant. The total number of the isozymes in the seedlings was 8 at both the altitudes of 700m and 2370m, and half the number of these isozymes were found to be main ones (Fig. 3). Main isozymes except for isozyme 1 and 11 had smaller pI value on 700m seedlings than 2370m ones, and this altitudinal difference in the mobility of main isozymes in the seedlings was similar to that in the mature plants.

A band for isozyme 1 in the seedlings was more wide than in the mature plants. A band for isozyme 11 in the seedlings was more wide and with a deeper colour than that for isozyme 11 in the mature plants. Although most of the isozymes in the mature plants and in the seedlings were varied on frequencies of appearance among the habitat altitudes, isozyme 2 and 11 in the mature plants and isozyme 1 and 11 in the seedlings were found in all the plants analyzed with a stable condition of staining.

Discussion

S. Virgaurea complex is distributed widely from basal to alpine zone in mountain area. TURESSON[20] has reported that *S. Virgaurea* growing in high altitudes is more dwarf and come into flower in earlier season than in low altitudes, and has suggested that these differences are genetical. BJÖRKMAN and HOLMGREN[2] have reported a morphological difference along with a physiological one of *S. Virgaurea* among different habitats on altitude. SANO and UJIHARA[18] have reported that an altitude of about 2000m was the border for a difference in flowering season for *S. Virgaurea* complex. KAWANO[8] has discussed ecotype differentiation of *S. Virgaurea* complex in various conditions of light and of habitat altitude. In these papers, it is remarked that morphological and ecological changes of *S. Virgaurea* complex with the increase of habitat altitude show remarkable occurrence at a level in subalpine zone. The morphological and ecological results obtained by the authors are identical to these results. Concerning these differences with the increase of habitat altitude in Japanease mountain area, KAWANO and TAKASU[9] have suggested that *S. Virgaurea* ssp. *leiocarpa* in the area above subalpine zone is differentiated from ssp. *asiatica* in the area below the zone as a result of adaptation to alpine climate.

On the isozymes in the mature plants on the eastern slope of Mt. Norikura, an altitudinal variation seems to be shown on main isozymes, except for isozymes 2 and 11, which are increased from 4 to 8 elements with altitudinal increase from 700m to 2200m alt., and then, are decreased to 5 elements at 2730m alt.. Furthermore, on main isozymes incresed on number with increasing altitude, these showing a high activity have a tendency to appear at higher pI values. Such variation in main isozymes is made more clear when the isozyme characteristic in the plants at 30m alt. on the Japan Sea side is compared with that on the eastern slope of Mt. Norikura, although the Japan Sea side is strictly isolated from the experimental area on Mt. Norikura and is under an Oceanic climate in contrast with an inland climate on Mt. Norikura. The variation in peroxidase isozyme polymorphism seems to support ABE and TAKASU's cytological data[1] that *S. Virgaurea* complex in various altitudinal habitats showed some morphological changes of chromosome at near 1850m alt..

An important characteristic of the isozymes in the mature plants is that the appearance or disappearance of a peroxidase isozyme among different altitudes involves a process of no, minor, and main isozyme or its reverse process, except for isozyme 10 which appeared unexpectedly at 2000m alt.. This process seems to explain the gradual increase or decrease of genetic variation with the altitudinal change of habitat. The decrease of the number of peroxidase isozymes at 2370m alt. may result from a genetic specialization like the gradual decrease of esterase isozyme polymorphism from the center to the periphery of rice plant distribution[14]. EIGA and SAKAI[5] have suggested the decrease of genetic variation from the middle to the lower and upper limits of vertical distribution on the bass of altitudinal variation in freezing resistance of *Abies sachalinensis*. Under these considerations, isozyme 10 may be minor at an altitude between 700m and 2000m alt..

Main isozymes in 700m and 2370m seedlings grown under controlled conditions are characterized by medium and large mobility, respectively, and such isozyme characteristics are similar to these found among the mature plants in different habitats on altitude. This fact suggests that the altitudinal change in main isozyme character on mobility has been differentiated already in the seedling age and is genetical.

On the isozyme compositions in the seedlings and in the mature plants at 700m and 2370m alt., isozyme 4 is not found only in the seedlings at 700m alt., and isozyme 3 is never found in either of the growth ages at 2370m alt.. These facts may suggest a possibility that isozyme 4 at 700m alt. is regulated in its function through the growth of *S. Virgaurea* ssp. *asiatica* while isozyme 3 is lacking from *S. Virgaurea* ssp. *leiocarpa* at 2370m alt.. For this reason, genetic differences on peroxidase between *S. Virgaurea* ssp. *asiatica* and ssp. *leiocarpa* seem to be shown on the presence or absence of isozyme 3 and on a different mobility of main isozymes.

It is possible to conclude that *S. Virgaurea* ssp. *asiatica* and ssp. *leiocarpa* in sunny places have many intraspecific variants along an altitudinal gradient, and that peroxidase isozymes which are most polymorphic at a distribution border of both the subspecies have a tendency to lower the polymorphism at altitudes below and above the border. These genetic variants may be developed through adaptayion to altitudinal climate. The activity change of an isozyme has been reported on a few kinds of plant with a relation to a factor in environmental conditions[4,7,10,15,16]. However, at present, the specific factor for peroxidase isozyme variation remains to be made clearly.

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