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The Intraspecific Differentiation of *Cardamine flexuosa*, Cruciferae, in Japan and Nepal

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**The Intraspecific Differentiation of *Cardamine flexuosa*, Cruciferae, in Japan and Nepal.
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The intraspecific differentiation of *Cardamine flexuosa*, a widespread weed collected in Japan and Nepal, was compared morphologically, ecologically and genetically. In the present study six biotypes growing parapatrically in paddy fields, gardens and a stone wall in Japan and Nepal were detected. The intraspecific differentiation of *C. flexuosa* in the process of adaptation to gardens, paddy fields, stone wall and so on has occurred independently in Japan and Nepal. A model of intraspecific differentiation of *C. flexuosa* is proposed within a limited comparison in Japan and Nepal.

Key Words : *Cardamine flexuosa*, Ecotype, Hybrid, Intraspecific differentiation, Weed

Introduction

The term "ecotype" was proposed for the ecological sub-unit of the product arising as a result of the genotypical response of an ecospecies to a particular habitat (Turesson, 1922, 1925). The ecotype is a basic biological entity. Its entity is adjusted to a range of habitats characterized by particular environments, e.g., annual fluctuations in climate, soils, other organisms and so on. The ecotypic differentiation is essentially physiologic-genetic (Clausen, 1967; Armbruster, 1985).

In widespread species, the ecogeographical differentiation is closely related to the size, migration, variation, selection, reproduction and isolation of populations (Ehrendorfer, 1968; Barton and Charlesworth, 1984). There are two extreme types of intraspecific differentiation: allopatric and partly sympatric patterns. The former pattern is found most typically in allogamous groups without restriction on intrapopulation gene flow, while the latter and more complex patterns are found in groups where intrapopulation gene flow is restricted (Ehrendorfer, 1968; Kawano et al., 1971; Kawano, 1974). The term "sympatric" differentiation has not been used to include such cases of differentiation on adjacent populations that are allopatric on the geographical criteria. Those adjacent populations are, therefore, described as parapatric (Jain and Bradshaw, 1966).

In a previous paper, the reproductive systems of four weedy *Cardamine* species,

Cruciferae, were compared (Kimata, 1983). As for the parapatric differentiation of *C. flexuosa*, the paddy field biotype was distinguished from the garden biotype by several characters in Japan. For example, the former biotype had longer reddish purple siliqua, narrow terminal leaflet of the uppermost leaf, more tillers and more hairy and deeper reddish purple stem than the latter. The differences were also detected in seed production, dispersal and germination modes, and in the rooting ability of stem segments.

In the present study the ecogenetical characters of *C. flexuosa* were compared to analyse the intraspecific differentiation in Japan and Nepal.

Materials and Methods

1) Materials

Seventeen strains of *Cardamine flexuosa* With. were used in the present study as shown in Table 1. Japanese strains were collected from five habitats: a garden, a stone wall, a roadside, a ditch and fallow paddy fields in three localities, Tokyo. Nepalese strains were collected from three habitats: a garden in a mountain area (Syangboche about 4000m alt.),

Table 1. Materials of *Cardamine flexuosa* used

| Cultivation no. | Generation | Locality | Habitat |
|-----------------|----------------|--|----------------------------|
| 83006 | | Syangboche, Nepal (3850 m alt.) | garden in mountain area |
| 83007 | | Katmandu, Nepal (1303 m alt.) | garden |
| 83009 | | Katmandu, Nepal | paddy field |
| 83010 | | Koganei, Tokyo, Japan (75 m alt.) | garden |
| 83011 | | Koganei, Tokyo, Japan | stone wall |
| 83012 | | Kunitachi, Tokyo, Japan (60 m alt.) | paddy field |
| 83001 | F ₂ | F ₁ 82001 | |
| 83002 | F ₂ | F ₁ 82003 | |
| 82001 | F ₁ | 83010 x 83012 (in 1981) | |
| 82003 | F ₁ | 83012 x 83010 (in 1981) | |
| site 1 | | parapatric population | roadside |
| 2 | | Kunitachi, Tokyo, | garden |
| 3 | | Japan | ditch |
| 4 | | | roadside |
| 5 | | | paddy field |
| 6 | | | paddy field |
| 7 | | | garden |

two gardens, one in Ratna Park and one at a hotel, and a paddy field (Katmandu about 1300m alt.). These plant specimens were placed on file with the Herbarium of Field Studies Institute for Environmental Education (FSIFEE), Tokyo Gakugei University.

2) Sampling materials of natural populations

In order to investigate the distribution of two biotypes of *C. flexuosa* in Kunitachi, Tokyo, the examination area was divided into 144 sections with 250m meshes. All sections were observed in April, 1979, and 1-5 plants each were collected from each of 35 sections where this species was found. The terminal leaflet width of the uppermost leaf and the length of the largest silique of 67 matured plants were measured.

Moreover, several plants were collected in April, 1984, from seven sites with different microenvironments in a section where two biotypes grew parapatrically. The terminal leaflet width and the length of the largest silique of these plants were measured similarly.

3) Genetic analysis by intraspecific crossing experiment

The young flowers of Japanese and Nepalese strains were bagged with paraffin-paper bags separately to prevent natural cross-pollination after castration. The intraspecific crossing among them was carried out 2-3 days latter. One month after crossing, the fructification rate (crossability) was calculated from the ratio of the number of fruiting siliques to the number of flowers. The self- and open-pollination rates were also calculated as described in the previous paper (Kimata, 1983).

In order to observe the seed germinating rate of F_1 hybrids, 100-200 seeds were sown on parlite soil in Petri dishes. This experiment was carried out under natural daylight (December 26, 1983 - February 21, 1984) in a greenhouse. The germinating rate was observed 57 days after sowing. The pollen fertility of F_1 hybrids was estimated on the basis of the stainability of about 100-200 pollen grains each from 1-20 flowers dipped in acetocarmine solution. Several morphological characters in about 3-20 plants from each strain were measured.

Using two F_2 hybrid populations (83001 and 83002) of reciprocal crosses between a Japanese garden biotype and a Japanese paddy field biotype, the segregation of six quantitative characters (e.g., silique length, plant height, number of tillers, width of terminal leaflet, stem hairiness and stem color) in 70 (83001) and 119 plants (83002) was measured in 1984.

Results

Distribution of two Japanese biotypes

As the result of the detailed field observations in Kunitachi, Tokyo, the paddy field biotype of *C. flexuosa* grew mainly in paddy fields in the Aoyagi Terrace and the flood plain of Tama River, 55-65 m alt., as shown in Fig. 1A. On the contrary, the garden biotype grew in shady gardens and on roadsides in the Tachikawa Terrace, 70-80 m alt., with the exception of one section (Fig. 1B). However, this biotype could not be found in densely built-up areas developed after 1960. In the exceptional section, these two biotypes grew closely or parapatrically in gardens, ditches, roadsides and paddy fields.

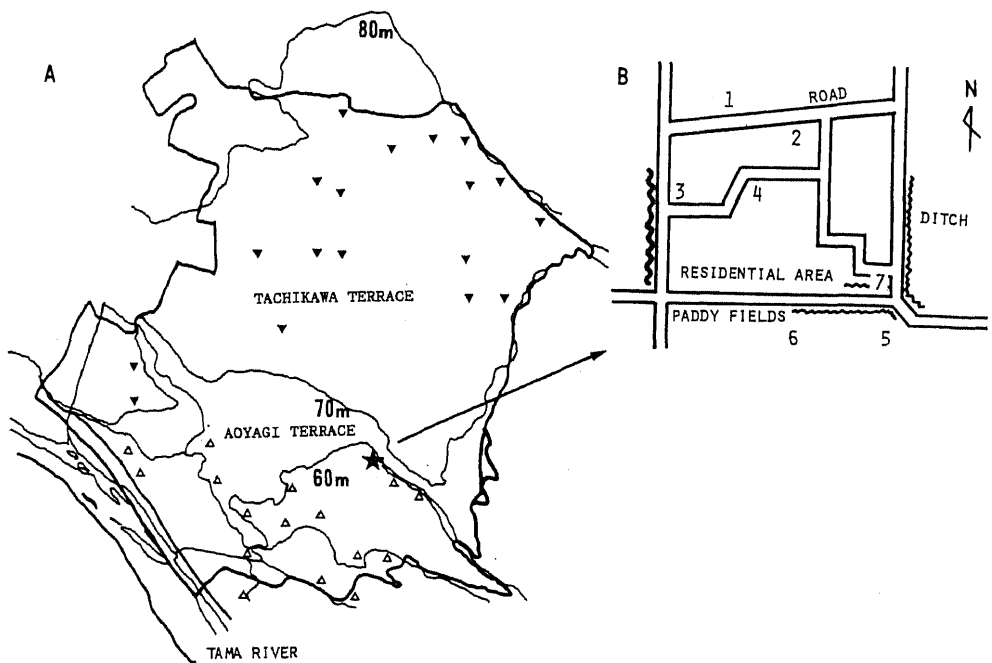


Fig. 1. Distribution of *Cardamine flexuosa* in Kunitachi, Tokyo.

A: △, paddy field biotype; ▼, garden biotype. B: parapatric zone; sites 1 and 2, garden; 3 and 7, ditch side; 4, roadside; 5 and 6, paddy field.

The two quantitative characters, the width of the terminal leaflet and the length of the largest silique of 36 plants from paddy fields in 16 sections and 31 plants from gardens and roadsides in 19 sections of Kunitachi were measured. The mean values of those two characters with their standard deviations in paddy field biotype and garden biotype are illustrated in Fig. 2. The mean and standard deviation of these characters in F_1 hybrid (82003) is also shown in Fig. 2. Moreover, values of those two characters in each of the plants that were growing at seven sites in a parapatric section are also drawn in Fig. 2. The two biotypes can be clearly distinguished from each other morphologically. For example,

the paddy field biotype has a narrower terminal leaflet and longer silique than the garden one. Their F_1 hybrid (82003) has intermediate characters between them. The plants growing at site 1 (garden) can be identified as the garden biotype, while the plants growing at sites 5 and 6 (paddy fields) can be identified as the paddy field biotype. However, the plants growing at sites 2, 3, 4 and 7 (garden, ditches and roadside) show wide morphological variations between these two biotypes.

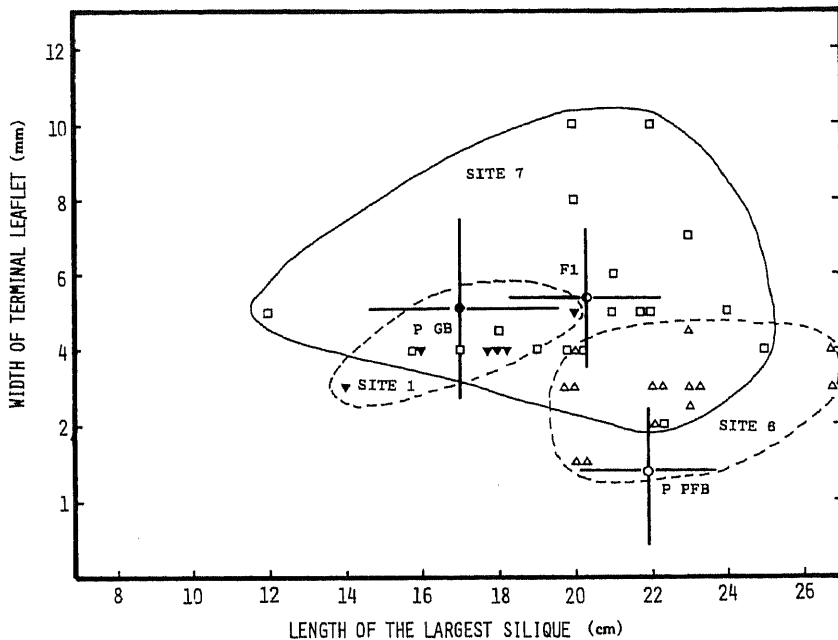


Fig. 2. Variations of the width of terminal leaflet and the length of the largest silique. ○, the mean and standard deviation (SD) in a paddy field biotype (P PFB, 83012) collected in Kunitachi; ●, the mean and SD in a garden biotype (P GB, 83010) collected in Koganei; ⊙, the mean and SD in F_1 hybrid (82003) of PFB and GB; ▼, garden biotype (site 1); △, paddy field biotype (site 6); □, parapatric population (site 7).

Segregation of six characters in F_2 hybrids

Some significant differences of characters were recognized between the paddy field biotype and the garden biotype in Japan (Kimata, 1983). The phenotypic segregation of four characters in two strains (83001 and 83002) of F_2 hybrid is observed as illustrated in Fig. 3. Those characters were quantitative and plastic.

The silique length of the garden biotype (18-21 mm) was shorter than that of the paddy field biotype (21-27 mm), although the latter showed large variability. Because most of their F_1 plants had siliques shorter than those of the paddy field biotype as shown in Fig. 3A, the short silique seemed to be dominant. The width of the terminal leaflet of the

garden biotype was wider than that of the paddy field biotype as shown in Fig. 3B. Their F_1 hybrid plants were also similar to the garden biotype. The plant height and the number of tillers of F_1 hybrid plants indicated remarkable hybrid vigor as shown in Fig. 3C and 3D. Frequency distributions of these four characters in two F_2 hybrid strains represented normal curves as shown in Fig. 3.

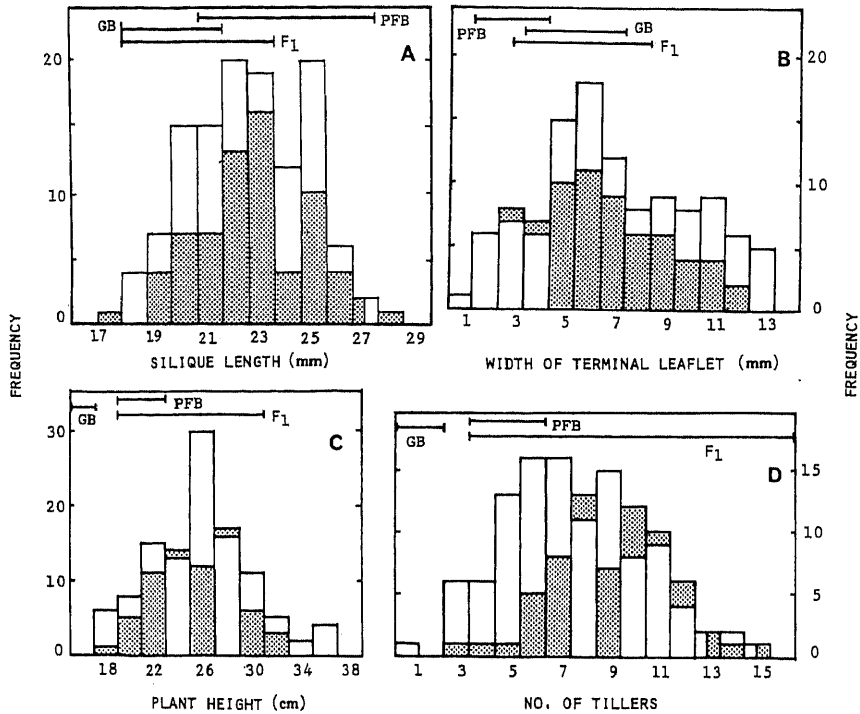


Fig. 3. Frequency distributions of four characters in F_2 hybrids. A, silique length; B, width of terminal leaflet; C, plant height and D, number of tillers. Shaded square, F_2 hybrid (83001) and open square, F_2 hybrid (83002). PFB, variation ranges of paddy field biotype (P); GB, those of garden biotype (P); F_1 , those of F_1 hybrid (82003).

The segregation of hairiness and coloration of the stem in these F_2 hybrid strains is shown in Table 2. The paddy field biotype had a reddish purple colored and hairy stem, while the garden biotype had a green colored and glabrous stem. Their F_1 hybrid had a reddish purple colored and hairy stem. Therefore, the reddish purple coloration and hairiness seemed to be dominant characters. Though the segregation proportions of these characters in two F_2 hybrid strains could not be explained by the presence of a single major gene in the chi square test.

Several characters of five strains

A comparison of ten characters in five strains from Japan and Nepal is shown in Table

Table 2. Phenotypic segregation (number of plants) of the hairiness and coloration of stem in F₂ hybrid plants

| Hairiness Coloration | glabrous green | | | dense reddish purple | Total |
|-------------------------|-------------------|----|----|-------------------------|-------|
| | ± | + | ++ | +++ | |
| 83001 | 14 | 13 | 32 | 10 | 69 |
| | 4 | 46 | 13 | 5 | 68 |
| 83002 | 15 | 13 | 34 | 51 | 113 |
| | 1 | 9 | 45 | 50 | 105 |

Table 3. Comparison of ten characters of five strains collected in Japan and Nepal

| Characters Habitat* | J a p a n | | | N e p a l | |
|-----------------------------------|----------------|-------------------|-------------------|------------|-------------|
| | 83012 PF | 83010 G | 83007 G | 83006 M | 83009 PF |
| Growth habit | winter ann. | year-long ann. | year-long ann. | ? | ? |
| Self-pollination rate (%) | 99.1 | 100 | 99.8 | 100 | 99.2 |
| Open-pollination rate (%) | 92.7 | 85.8 | 98.6 | 100 | 99.2 |
| Plant height (cm) | 21.7 | 15.4 | 21.5 | 24.0 | 24.1 |
| No. of tillers | 4.7 | 2.1 | 11.9 | 9.0 | 8.0 |
| Silique length (cm) | 21.4 | 20.6 | 22.3 | 22.0 | 21.1 |
| Width of terminal leaflet (mm) | 2.2 | 5.8 | 5.1 | 7.5 | 7.7 |
| Stem color | RP | GR | RP | RP | GR |
| Hairiness of stem | ++ | ± | +++ | ++ | ± |
| Plant form | tufted | branched | tufted | branched | branched |

*PF, paddy field; G, garden; M, mountain.

**RP, reddish purple; GR, green.

3. The growth habit of 83012 (growing in a paddy field) was a winter annual, while those of 83010 and 83007 (growing in a garden) were year-long annuals. The growth habit of 83009 (growing in a paddy field, Nepal) and 83006 (growing on mountain area, Nepal) could not be checked in their natural habitats.

The self- and open-pollination rates were also more than 85% in every strain, so that all strains were self-pollinated. The plant height in 83010 was the smallest among all strains observed. The number of tillers (11.9) in 83007 was six times as many as in 83010. That(4.7) in 83012 was twice as many as in 83010. Those for 83006 and 83009 were approximately the same.

The silique length in all strains was about 21 mm. The width of the terminal leaflet in 83009 was the broadest of all, while that in 83012 was the narrowest. Those for 83010 and 83006 were similar to 83007 and 83009, respectively. The stem color in both 83009 and 83010

was green, but the others were reddish purple. Three strains, 83010, 83007 and 83006 had densely-haired stems, but 83009 and 83010 had glabrous stems. The plant form of 83012 and 83007 was tufted, while that of 83009, 83010 and 83006 was branched. Because these five strains can be classified easily using these diagnostic characters, each strain may be called a biotype.

Crossability among six biotypes

The crossability among six biotypes in Japan and Nepal (12 cross combinations) and the seed germination rate and pollen fertility of their F₁ hybrids are shown in Fig. 4.

The crossability of both a Japanese garden biotype x a Japanese paddy field biotype (83010x83012) and the reciprocal was 92.3-100%. The seed germination rate and pollen fertility of their F₁ hybrids were 72.4-81.5 and ca. 90%, respectively. On the other hand,

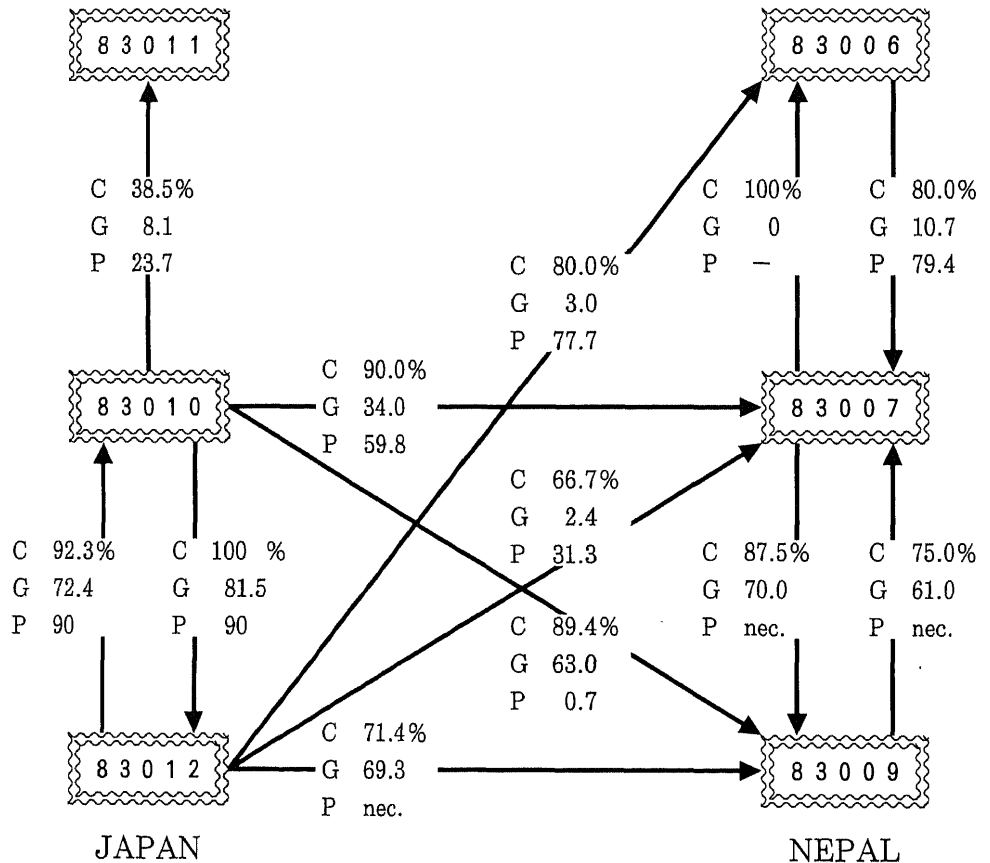


Fig. 4. Crossability among six strains from Japan and Nepal and the seed germination rate and pollen fertility of their F₁ hybrids. C, crossability(%); G, seed germination rate(%) and P; pollen fertility(%). nec, seedlings died by necrosis; the direction of arrow, pollen flow by artificial crossing.

those of a stone wall biotype x a garden biotype (83011x83010) were 38.5, 8.1 and 23.7%, respectively. Between Nepalese biotypes, the crossability of both a garden biotype x a paddy field biotype (83007x83009) and the reciprocal and the seed germination rate of their F_1 hybrids were 75.0-87.5 and 61.0-70.0%, respectively, but the seedlings of F_1 hybrids died by necrosis. The crossability of a mountain biotype x a garden biotype (83006x83007) and the reciprocal was 80.0-100%. While seeds of the former F_1 hybrid did not germinate, the seed germination rate and pollen fertility of the latter hybrid were 10.7 and 79.4%, respectively.

Between Nepalese and Japanese biotypes, the crossability of a Nepalese garden biotype x a Japanese garden biotype (83007x83010) was 90.0%. The germination rate and pollen fertility of the F_1 hybrid were 34.0 and 59.8%, respectively. However, those of a Nepalese paddy field biotype x a Japanese garden biotype (83009x83010) were 89.4, 63.0 and 0.7%, respectively. The crossabilities of a Nepalese mountain biotype x a Japanese paddy field biotype (83006x83012), a Nepalese garden biotype x a Japanese paddy field biotype (83007x83012) and a Nepalese paddy field biotype x a Japanese paddy field biotype (83009x83012) were 66.7-80.0%. The seed germination rates of the F_1 hybrids in the 1st and 2nd cross combinations were only 2.4-3.0%, while that of the 3rd one was 69.3%. However, the pollen fertilities of the F_1 hybrids in the 1st and 2nd cross combination were 31.3-77.7%, while seedlings of the F_1 hybrid in the 3rd one died by necrosis.

Discussion

The genetic basis of ecotypic divergence is likely to be very complex. For example, in a cross between two ecotypes of *Plantago lanceolata*, F_2 individuals carrying a majority of allozyme alleles from a hayfield population performed better when transplanted to a hayfield than did those with a majority of alternative alleles from a pasture population. On the contrary in the pasture site, plants with a majority of pasture-derived alleles performed better. Adaptive differentiation between the two ecotypes apparently segregated at many loci scattered throughout the genome as already suggested (Wolff, 1988; Frankel et al., 1995).

Ecotypic differentiation as a process of adaptation to a paddy field (or moist environment) has been reported in several species. An early ecotype of *Agropyron tsukushiense* (Honda) Ohwi var. *transiens* (Hack.) Ohwi is found in winter fallow paddy fields (Sakamoto 1961; Kimata and Sakamoto, 1982). Compared with the common type of this species often found in fields and on roadsides, the early ecotype has many contrasting characters: shorter spikes, larger and heavier seeds, smaller phenotypic variation, and earlier maturity. In *Alopecurus aequalis* Sobol. the lowland ecotype grows in winter fallow paddy fields, while the upland ecotype grows in upland fields (Matsumura, 1967). Compared with the upland ecotype, the lowland ecotype has several different characters: larger and heavier seeds, higher self-fertility, earlier maturity, and somewhat lower inherent seed dormancy.

Veronica peregrina L. is observed in the center and periphery of vernal pools (Linhart,

1974). Compared with the plants growing around the periphery, those at the center have many different characters: fewer but larger seeds, smaller phenotypic variation, more rapid and uniform seed germination, somewhat early maturity, and more biomass allocation into seeds.

The present study is concerned with the intraspecific differentiation of widespread species, *Cardamine flexuosa*. Moreover, it is interesting to compare the intraspecific differentiations occurring in Japan (the eastern border of Sino-Japanese Region) and in Nepal (near the western border of the Region).

Considerable differences in the intraspecific crossability observed among different combinations of biotypes indicate the occurrence of intraspecific differentiation among Japanese *C. flexuosa* strains. The intraspecific differentiation has already been suggested by several morphological and ecological characters (Table 3 and Fig. 2), such as growing season, mode of seed dispersal, germination period, flowering period, and others (Kimata, 1983). The winter annual paddy field biotype can establish large populations only in the restricted habitats, such as the winter fallow paddy fields as shown in Fig.1. On the other hand, the year-long annual garden biotype, which produces fewer seeds can establish small populations (a few plants per m²) in rather wet microenvironments of various habitats, such as gardens, roadsides, and nurseries. As shown in Fig. 2, their F₁ hybrid has intermediate characters between the paddy field biotype and the garden biotype in Japan. Site 7 (a large population) is a parapatric habitat for both biotypes, and the plants growing here show wide variation in morphological and ecological characters, which suggests the occurrence of hybridization between these two biotypes.

The annual growth habit is classified into three different types: summer, winter and year-long annual (Kimata and Sakamoto, 1979). The genus *Cardamine* usually grows in winter and flowers in spring. The year-long annual type might be derived from a winter annual type. Therefore, the year-long annual garden biotype establishes small populations in somewhat unstable habitats twice in summer and winter.

Various patterns of phenotypic segregation of the six quantitative characters of Japanese F₂ hybrids shown in Table 2 and Fig. 3 and a comparison of ten characters between the paddy field biotype and the garden biotype shown in Table 3 clearly indicate the occurrence of intraspecific differentiation in *C. flexuosa*. The plant height of paddy field biotypes is taller than that of garden biotypes both in Japan and Nepal. However, on the six other characters (number of tillers, silique length, width of terminal leaflet, stem color, hairiness of stem and plant form), the variation patterns of Japanese biotypes show the opposite trend to those of Nepalese biotypes. Therefore, the intraspecific differentiation of *C. flexuosa* in Japan did not occur in parallel with that in Nepal.

The intraspecific differentiation of *C. flexuosa* has occurred in many directions as shown in Fig. 4. According to the crossability among strains examined, the garden biotype (83010) is shown to be isolated sexually from the stone wall biotype (83011), but not from the paddy field biotype (83012) in Japan. The garden biotype (83007) is considered to be isolated sexually from the paddy field biotype (83009) in Nepal because of the necrosis of F₁ hybrid seedlings. As F₁ hybrid seedlings become necrosis, the Japanese paddy field

biotype (83012) is also considered to be isolated from the Nepalese paddy field biotype (83009). As the other hand, the Japanese garden biotype (83010) is not considered to be isolated from the Nepalese garden biotype (83007), which itself has not been isolated from the mountain biotype (83006). As the results of crossings between the Japanese paddy field biotype (83012) and the Nepalese garden biotype (83007), and between the Japanese garden biotype (83010) and the Nepalese paddy field biotype (83009), occurrence of sexual isolation among them is also indicated. The Japanese paddy field biotype (83012) is not very isolated, yet, from the Nepalese mountain biotype (83006). Therefore, each garden biotype in Japan and Nepal is the prototype among six biotypes and can be called a garden "ecotype." Turesson (1922, 1925) defined the term "ecotype" as the product of the genotypical reaction of ecospecies to a particular, defined set of environment conditions. He also defined the sub-taxon "ecophene" as the reaction-type to a unique microenvironment.

Within a limited comparison of Japan and Nepal carried out in the present study, a model of intraspecific differentiation of *C. flexuosa* is shown in Fig.5. The garden ecotype may be an original biotype because neither garden biotype is isolated from the other. It is a very interesting fact that there is no sexual isolation between the garden ecotype and the paddy field ecophene in Japan, but not between the garden ecotype and the paddy field ecotype in Nepal. As observed in other weeds growing in paddy fields (Sakamoto, 1961; Matsumura, 1967; Linhart, 1974), the Japanese paddy field ecophene of *C. flexuosa* shows some phenotypic variation in various characters. This fact indicates a great diversity of intraspecific differentiation in *C. flexuosa* which may correlate with the period when rice cropping in paddy fields dispersed throughout Asia. The stone wall ecotype (83011)

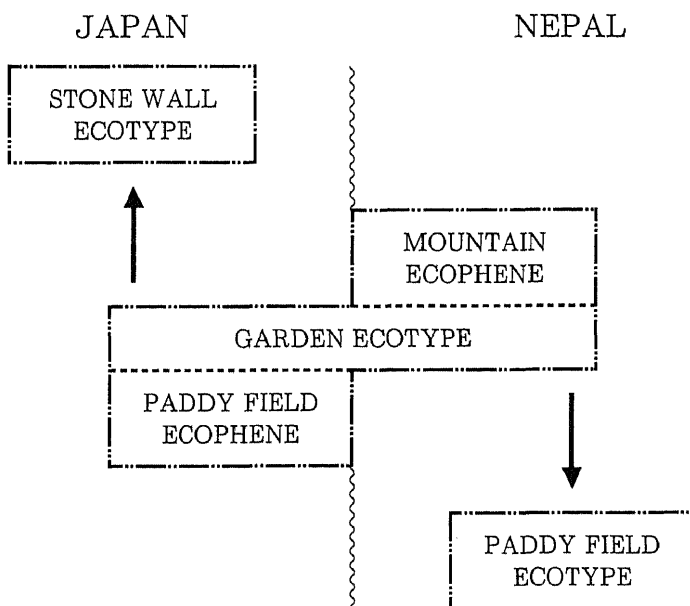


Fig. 5. A model of intraspecific or ecotypic differentiation in Japan and Nepal.

growing on a stone wall in Koganei, Tokyo is different morphologically, ecologically and genetically from the garden ecotype (83010). The Nepalese garden ecotype includes the mountain ecophene in Nepal. The intraspecific or ecotypic differentiation has occurred independently in Japan and Nepal.

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References

- Armbruster, W.S. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution* 39:733-752.
- Barton, N.H. and B. Charlesworth. 1984. Genetic revolutions, founder effects, and speciation. *Ann. Rev. Ecol. Syst.* 15: 133-164.
- Clausen, J. 1967. Biosystematic consequences of ecotypic and chromosomal differentiation. *Taxon* 16:271-279.
- Ehrendorfer, F. 1968. Geographical and ecological aspects of infraspecific differentiation. In: V. H. Heywood, ed., *Modern Methods in Plant Taxonomy* pp. 261-296. Academic Press, London.
- Frankel, O.H., A.H.D. Brown and J.J. Burdon. 1995. *The conservation of plant biodiversity*. Cambridge University Press, Cambridge.
- Jain, S.K. and A.D. Bradshaw. 1966. Evolutionary divergence among adjacent plant populations. *Heredity* 21:407-441.
- Kawano, S. 1974. *Evolutionary Biology in Plants. II. Speciation and Adaptation*. Sanseido, Tokyo (in Japanese).
- Kawano, S., M. Suzuki and S. Kojima. 1971. Biosystematic studies on *Maianthemum* (Liliaceae-Polygonatae). V. Variation in Gross Morphology, Karyology, and Ecology of North American Populations of *M. dilatatum sensu lato*. *Bot. Mag. Tokyo* 84:299-318.
- Kimata, M. 1983. Comparative studies on the reproductive systems of *Cardamine flexuosa*, *C. impatiens*, *C. sucutata*, and *C. lyrata*, Cruciferae. *Bot. Mag. Tokyo* 96:299-312.
- Kimata, M. and S. Sakamoto. 1979. Comparative studies on the population dynamics of *Mazus japonicus* and *M. Miquelii*, Scrophulariaceae. *Bot. Mag. Tokyo* 92:123-234.
- Kimata, M. and S. Sakamoto. 1982. Interrelationships between the mode of reproduction and the habitat of two weedy *Agropyron* species, *A. tsukushiense* and *A. humidorum*, Gramineae. *Weed Research (Japan)* 27:103-111.
- Linhart, Y. B. 1974. Intra-population differentiation in annual plants. I. *Veronica peregrina* L. raised under non-competitive conditions. *Evolution* 28:232-243.
- Matsumura, M. 1967. Genecological studies on the foxtail grass, *Alopecurus aequalis*, in

- Japan. Res. Bull. Fac. Agr., Gifu Univ. 25:129-208 (in Japanese).
- Sakamoto, S. 1961. An early ecotype of *Agropyron tsukushiense* var. *transiens*. *Seiken Ziho* 12:45-58.
- Turesson, G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* III:211-350.
- Turesson, G. 1925. The plant species in relation to habitat and climate — Contributions to the knowledge of genecological units. *Hereditas* VI:147-236.
- Wolff, K. 1988. Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. 3. Natural selection in an F₂ population. *Theoretical and Applied Genetics* 75: 772-778.