カワラヨモギの「ほふく型」と「直立型」の開花日，頭花サイズ，頭花中の利胆成分含量及び分子レベルの相違について

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The differences of flowering date, flower head size, choleretic substances contents and molecular characters between erect- and prostrate-growth forms of *Artemisia capillaris* in Japan

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Abstract

The capitulum of *Artemisia capillaris* has been used for medicinal purpose. Growth form of this species seems to be different among the plants found in riverbed and coastline but no clear report in this regard is available. A comparative study was, therefore, carried out among the plants collected from eighteen locations covering riverbed and coastline to elucidate the differences in growth form, flowering date, flower head size and choleretic substances (capillarisin and 6,7-dimethylesculetin) in flower head as well as molecular characters. For supplement of data, growth form and flower head size of sixty two herbarium specimens were also examined. Results revealed that, with a few exceptions, the plants collected at riverbed showed the erect-type of growth form, while the plants collected at coastline showed the prostrate-type of growth form. These growth forms were easily distinguishable and sustained over years of cultivation. Concerning the other characters, no clear difference was found between erect- and prostrate-types in flowering date. However, at early flowering stage, both types were discriminated by flower head size and its shape. Moreover, the content of 6,7-dimethylesculetin in erect-type was significantly higher than that in prostrate-type and a similar trend was also found in content of capillarisin. From these findings, it is considered that each growth form depends on genetic nature and can be regarded as an ecotype. The results of cluster analysis using DIG-RAPD divided all the used plants into two groups according to the plants collected from riverbed or coastline, while it was not correlated with other characters.

Key words: *Artemisia capillaris*, growth form, flower head size, choleretic substances, DIG-RAPD.

Introduction

Generally, physiological as well as morphological characters of plants vary depending on environmental conditions in their habitats (Matsumura 1967 ; Tominaga et al. 1989 b). Oyama (1993) in *Arabis serrata*, and Yamanishi and Fukunaga (1983) in *Plantago asiatica* suggested that such variations in physiological and morphological characters among the plants grown under different environmental conditions in habitats occurred due to different kinds of selection pressure and degree of its intensity.

Artemisia capillaris Thunb. is a perennial herb of the family Compositae distributed in

East Asia (Kitamura et al. 1957). The capitulum of this plant is listed as crude drug named "Artemisiae capillaris Flos" in the Japanese Pharmacopoeia XIII (Ministry of Health and Welfare 1996) and used as an anti-inflammatory, antipyretic and diuretic for liver and jaundice in Chinese medicines (Yamahara et al. 1982; Okuno et al. 1983; Kiso et al. 1984). The substances i. e., capillarisin (CAP) and 6,7-dimethylesculetin (DME) were isolated from the capitulum for choleretic effects (Yamahara et al. 1989; Kitagawa et al. 1983; Okuno et al. 1988) and were considered as important constituents of crude drug. The Artemisiae capillaris Flos is mostly collected from the plants grown in their native habitats in Japan. As a result, the crude drug dealt in Japanese crude drug market morphologically varied with geographic origins (Namba et al. 1974).

In Japan, except Hokkaido, *A. capillaris* is usually distributed along the riverbed and coastline (Kitamura 1940; Ohwi 1992). Kitamura (1940) reported that this species grown in Japan exhibited great variation of growth form, where the plants grown at riverbed were erect and those at coastline were prostrate in growth form. In addition, our observation revealed that these differences of growth form are easily visible and sustain over years of cultivation. Thus, it may be assumed that such difference of environmental conditions in location as riverbed or coastline could influence not only the growth form but also the physiological and chemical characters in *A. capillaris*. However, no comprehensive study has yet been made to discern this obvious difference between erect- and prostrate-types found in riverbed and coastline in chemical as well as molecular characters. Therefore, in this report we examined differences in flowering date, flower head size and contents of CAP and DME between erect- and prostrate-types of *A. capillaris* which were collected from eighteen locations covering riverbed and coastline in Japan. For supplement of data, growth form and flower head size of sixty two herbarium specimens were also examined. In addition, genetic polymorphism of those two types was analyzed by highly sensitive randomly amplified polymorphic DNAs by digoxigenin labeling (DIG-RAPD).

Materials and methods

Materials

Seeds or seedlings of *A. capillaris* were collected from eighteen locations (latitude lies between 40° 57′ N to 30° 32′ N) covering riverbed and coastline in Japan during 1995 to 1997 (Table 1, Fig. 1) and were cultivated in the field of Tsukuba Medicinal Plant Research Station (Lat.: 36° 01′ N, Long.: 140° 04′ E), National Institute of Health Sciences (TNIHS), Iba-

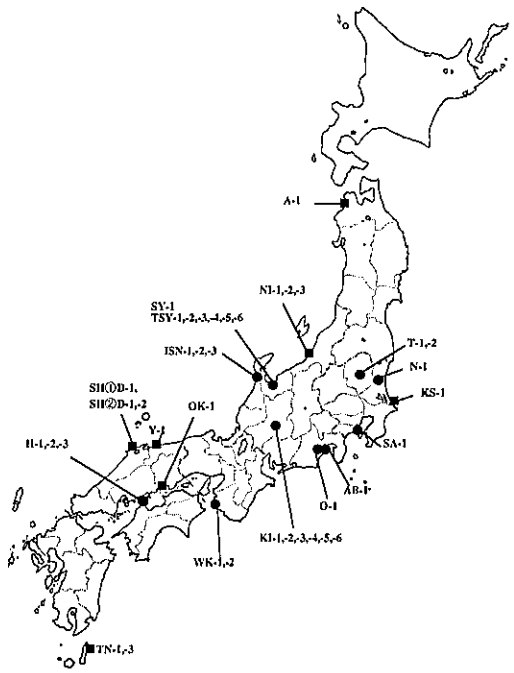


Fig. 1. Habitats of *Artemisia capillaris* collected at eighteen different locations in Japan. Black spots and black squares are marked with the station of erect- and prostrate-types, respectively.

raki, Japan until comparative experiment in 1997. The plants collected from each location are arbitrarily designated as accession in this experiment. All accessions were successively maintained in TNIHS (Introduced numbers of each accession in TNIHS are as follows; Erect-type, ISN: 97-491; H: 95-363; SY: 96-406; TSY: 95-357; T: 95-365; N: 95-360; SA: 95-362; KI: 95-356; O: 95-358; AB: 97-488; WK: 95-359. Prostrate-type, A: 96-407; NI: 95-361; KS: 95-364; Y: 97-490; SH①D: 97-492; SH②D: 97-493; OK: 97-489; TN: 95-355). During cultivation, growth form of each

Table 1. List of *Artemisia capillaris* of forty two individuals collected at eighteen different locations in Japan

Location		Latitude[1)]	Abbreviation	
collected at coastline				
Shariki, Nishitugaru, Aomori Pref.	Nishitugaru, Coastline	40° 57′	A-1	**
Kakizaki, Nakakubiki, Niigata Pref.	Kakizaki, Coastline	37° 15′	NI-1	**
			NI-2	**
			NI-3	**
Takamatsu, Kahoku, Ishikawa Pref.	Coastline	36° 46′	ISN-1	**
			ISN-2	**
			ISN-3	**
Hazaki, Kashima, Ibaraki Pref.	Kashima, Coastline	35° 48′	KS-1	**
Oosinozu, Yonago, Tottori Pref.	Coastline	35° 25′	Y-1	*
Taisha, Hikawa, Shimane Pref.	Hinomisaki, Coastline	35° 23′	SH①D-1	**
			SH②D-1	**
			SH②D-2	**
Kojima, Kurashiki, Okayama Pref.	Coastline	34° 32′	OK-1	*
Oosaki, Toyota, Hiroshima Pref.	Nakakushi, Coastline	34° 14′	H-1	*
			H-2	*
			H-3	*
Nakatane, Kumage, Kagoshima Pref.	Tanegashima, Coastline	30° 32′	TN-1	*
			TN-3	*
collected at riverbed				
Takaoka, Toyama Pref.	Shogawa, Riverbed	36° 44′	SY-1	**
Daimon, Imizu, Toyama Pref.	Shogawa, Riverbed	36° 42′	TSY-1	*
			TSY-2	*
			TSY-3	*
			TSY-4	*
			TSY-5	*
			TSY-6	*
Utsunomiya, Tochigi Pref.	Kinugawa, Riverbed	36° 34′	T-1	**
			T-2	**
Omiya, Kuji, Ibaraki Pref.	Nakagawa, Riverbed	36° 34′	N-1	**
Sagamihara, Kanagawa Pref.	Sagamigawa, Riverbed	35° 32′	SA-1	**
Kawashima, Hashima, Gifu Pref.	Kisogawa, Riverbed	35° 21′	KI-1	*
			KI-2	*
			KI-3	*
			KI-4	*
			KI-5	*
			KI-6	*
Nakakawane, Haibara, Shizuoka Pref.	Oigawa, Riverbed	35° 08′	O-1	*
			O-2	*
Ushizuma, Shizuoka, Shizuoka Pref.	Abekawa, Riverbed	34° 58′	AB-1	**
			AB-2	**
			AB-3	**
Gobo, Wakayama Pref.	Hidakagawa, Riverbed	33° 52′	WK-1	**
			WK-2	**

1) Geographical Survey Institute (1997).
*: Seeds collected from several plants at their habitats were sown in the field of TNIHS, and then numbers were given to the seedlings.
**: Numbers were given to the seedlings collected at their habitats.

individual was observed.

The sixty two herbarium specimens deposited in the Herbarium, Department of Botany, National Science Museum (TNS), Ibaraki, Japan were also used for observation of growth form and measurement of flower head size (Fig. 2, Appendix).

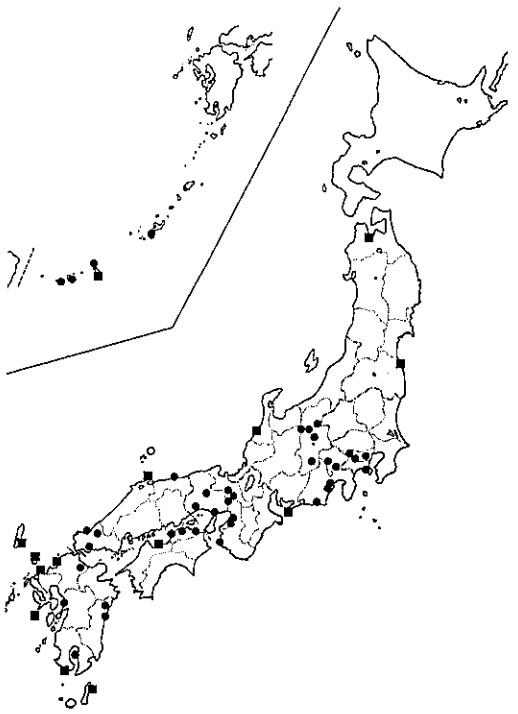


Fig. 2. Location of collection of herbarium specimens of *Artemisia capillaris* deposited in the Herbarium, Department of Botany, National Science Museum, Ibaraki, Japan.
Black spots and black squares are marked with the station of erect- and prostrate-types, respectively.

Field observation

One to six individuals that showed excellent growth in the field of TNIHS were selected from each accession (total forty two individuals) (Table 1, Fig. 1). Growth form and flowering date of each individual were recorded from August to October in 1997. The flowering date was defined as the first pollen diffusion date of each individual. Out of forty two individuals, thirty six individuals flowered and were used for measuring flower size and quantitative analysis of CAP and DME.

Measuring of flower head size of plant cultivated in the field and herbarium specimens

At day ten to fourteen after flowering, flower heads of each individual cultivated in the field of TNIHS were collected, dried at 40℃ for 48 hours and used to determine flower head size, and CAP and DME contents. The length and width of ten randomly selected dried flower heads were measured by a micrometer attached to a stereoscope (LEICAM 651) and the length/width ratio was calculated to evaluate the shape of flowerhead.

For supplement of above data, growth form and flower head size of sixty two herbarium specimens deposited in TNS were also examined. The growth form of each herbarium specimen was recorded, and then length and width of flower head were measured by the same methods mentioned above. As flower head size varies with flowering stages, flowering stage of each flower head was also recorded. Four flowering stages were arbitrarily defined as follows. (Stage 1: Involucral bracts begin opening and ray flowers release; Stage 2: Ray flowers extend, Disk flowers bloom and pollen diffuse; Stage 3: Disk flowers fully bloom and their stigmas elongate; Stage 4: Corollas drop, seeds in disk flowers mature and involucral bracts remain).

Quantitative analysis of CAP and DME in flower head

After measuring the length and width of flower head obtained in the field of TNIHS, the contents of CAP and DME in these flower heads were determined by the methods described by Ikenaga et al. (1989) as follows; ca. 500 mg of milled capitulum was extracted twice with 40 ml of 70% MeOH (ultrasonic 20 min), and then the combined solution made up to 100 ml with 70% MeOH. After filtration, an aliquot of sample solution was subjected to high performance liquid chromatography (HPLC). Analysis was performed under following conditions; HPLC system, D-6000, Hitachi; column, Develosil ODS column P-5 (4.6 mm i.d. ×250 mm, Nomura Chemical); mobile phase, H_2O : CH_3CN : H_3PO_4 = 720 : 280 : 1; monitored wave length, UV 292 nm. The standard regression lines and correlation co-

efficient between the amount injected volume of CAP and DME and their peak heights were obtained by using a standard solution for both CAP (0.0102 to 0.2035 mg/ml, r = 0.99) and DME (0.0088 to 0.1760 mg/ml, r = 0.99).

Highly sensitive random amplified polymorphic DNA by digoxigenin labeling (DIG -RAPD)

Out of eighteen accessions cultivated in the field of TNIHS, one flowering individual from each of fifteen accessions was selected and used for the molecular genetic analysis. The extraction of total DNA was carried out by ISOGEN (Nippon Gene(TM)) according to the protocol supplied by the manufacturer (Chomczynski 1993). The conditions and methods of polymerase chain reaction and polyacrylamide gel electrophoresis were carried out as described by Afele et al. (1996). The sequences of oligonucleotide primers having an arbitrary sequence at their 5′ terminal by labeling with digoxigenin-dideoxyuridine-triphosphate (DIG-ddUTP) used in this analysis were as follows : RA 1 ; 5′ - GTCTGACGGT-3′, RA 3 ; 5′ - CGATCGAGGA-3′, RA 5 ; 5′ - AAGCAGCAAG-3′, RA 7 ; 5′ - AGCACTTCGG-3′, RA 13 ; 5′ - TATTGTCAGC-3′, RA 14 ; 5′ - CGCGATTTGA-3′ and RA 16 ; 5′ - CCGACAGCTT-3′ (Monna et al. 1994). PCR products were transferred from polyacrylamide gels to nylon membranes (BOEHRINGER MANNHEIM) and immunologically detected by DIG Labeling and Detection Kit Nonradioactive (BOEHRINGER MANNHEIM) according to the protocol described by Tabei et al. (1996). Cluster analysis of these DIG-RAPD markers from the fifteen individuals was performed by using a standard statistical procedure (STATISTICA, Stat Soft). All bands as the position were regarded as the same DIG-RAPD markers, and a total of 199 markers were recorded from fifteen plants. The presence or absence of amplified DNA fragment was treated as a binary character. A dendrogram was constructed by STATISTICA (Stat Soft) using the unweighted pair group method with arithmetic means (UPGMA).

Results

Growth form

Growth form of *Artemisia capillaris* was remarkably different between the riverbed and coastline accessions observed in the field of TNIHS for two years. Based on the growth form, forty two individuals collected from eighteen locations could easily be classified into two types by visible observation i.e., the erect-type having relatively long erect straight main axes with assurgent branches and the prostrate-type having relatively short main axis with branches grown flat along the ground (Fig. 3). It was clear that all the accessions collected from the riverbed showed erect-type of growth form, while out of nine accessions collected from coastline, seven accessions showed prostrate-type and two accessions (ISN and H accessions) showed erect-type of growth form (Table 2).

Results of our observations on herbarium specimens deposited in TNS show that sixty two specimens could be allocated to forty eight erect- and fourteen prostrate-types. All prostrate-types were collected at coastline or near beach, while erect-type were collected at various locations, i.e., riverbed, disturbed habitats along roadway and mountain trail and near beach. No correlation was shown between growth form and latitude of their location of collection.

Flowering date

The flowering date of plants cultivated in the field of TNIHS is shown in Table 2. Out of forty two individuals, six prostrate-type individuals (NI-2, NI-3, SH①D-1, SH②D-1, SH②D-2 and Y-1) collected at coastline did not bloom, while thirty six individuals bloomed with a wide range of time (Table 2). Among individuals, T-1 and T-2 showed the earliest flowering date (Aug. 25), while TN-1 and TN-3 were the latest (Oct. 30). A sixty seven day difference of flowering date was found between the earliest flowering date and the latest one. Variations in flowering date were observed among the individuals in three accessions. In NI accession, NI-1 bloomed on Sept. 10, but the remaining individuals (NI-2 and NI-3) did not at all. In TSY and AB accessions, fourteen day differences was found between early flowering date and the latest one.

Fig. 3. Two growth form types of *Artemisia capillaris* collected in Japan. Erect-type (left) and prostrate-type (right).

Table 2. Growth form and flowering date of *Artemisia capillaris* of forty two individuals collected at eighteen different locations in Japan

Accessions[1]	Growth form	Flowering date (in 1997)[2]	Accessions[1]	Growth form	Flowering date (in 1997)[2]
collected at coastline			collected at riverbed		
A-1	Prostrate	Aug. 29	SY-1	Erect	Sep. 5
NI-1	Prostrate	Sep. 10	TSY-1	Erect	Sep. 10
NI-2	Prostrate	*	TSY-2	Erect	Sep. 3
NI-3	Prostrate	*	TSY-3	Erect	Sep. 3
ISN-1	Erect	Sep. 19	TSY-4	Erect	Sep. 10
ISN-2	Erect	Sep. 19	TSY-5	Erect	Sep. 17
ISN-3	Erect	Sep. 19	TSY-6	Erect	Sep. 3
KS-1	Prostrate	Sep. 1	T-1	Erect	Aug. 25
Y-1	Prostrate	*	T-2	Erect	Aug. 25
SH①D-1	Prostrate	*	N-1	Erect	Sep. 15
SH②D-1	Prostrate	*	SA-1	Erect	Sep. 1
SH②D-2	Prostrate	*	KI-1	Erect	Sep. 5
OK-1	Prostrate	Sep. 17	KI-2	Erect	Sep. 5
H-1	Erect	Sep. 16	KI-3	Erect	Sep. 10
H-2	Erect	Sep. 16	KI-4	Erect	Sep. 10
H-3	Erect	Sep. 16	KI-5	Erect	Sep. 5
TN-1	Prostrate	Oct. 30	KI-6	Erect	Sep. 10
TN-3	Prostrate	Oct. 30	O-1	Erect	Sep. 17
			O-2	Erect	Sep. 17
			AB-1	Erect	Sep. 10
			AB-2	Erect	Sep. 19
			AB-3	Erect	Sep. 5
			WK-1	Erect	Sep. 11
			WK-2	Erect	Sep. 19

1) Abbreviations are shown in Table 1.
2) defined the flowering date in each plant as the pollen diffusion.
*: no flower in 1997.

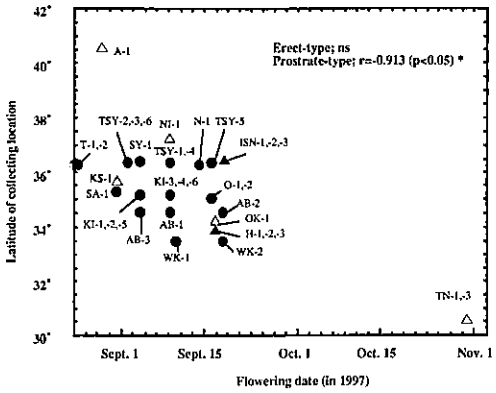


Fig. 4. Correlation between flowering date and latitude of collecting location of thirty six individuals of *Artemisia capillaris* collected at sixteen different locations in Japan.
● : Erect-type collected at riverbed ; ▲ : Erect-type collected at coastline ; △ : Prostrate-type collected at coastline.
* and ns indicate significant correlation between flowering date and latitude of collecting location and nonsignificant, respectively ($P<0.05$, correlation coefficient).
Abbreviations are shown in Table 1.

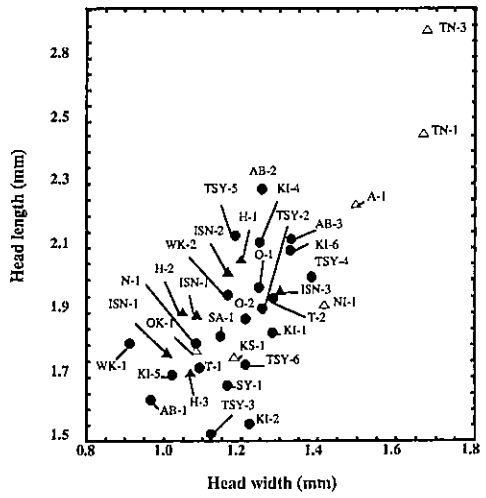


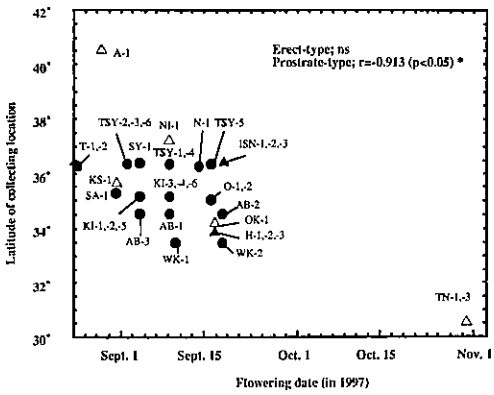
Fig. 5. Flower head size of thirty four indibiduals of *Artemisia capillaris* collected at sixteen different locations in Japan.
● : Erect-type collected at riverbed ; ▲ : Erect-type collected at coastline ; △ : Prostrate-type collected at coastline.
Abbreviations are shown in Table 1.

The flowering date in the prostrate-type had close relationship to latitude of their location of collection ($P<0.05$, $r=-0.913$), where individuals collected from higher latitude flowered earlier than those from lower latitude (Fig. 4). On the other hand, in the erect-type, no such relationship was observed between the flowering date and the latitude of their location of collection ($P<0.05$, $r=-0.304$) (Fig. 4). No significant difference in flowering date between erect- and prostrate-types was observed in this experiment.

Flower head size

The length and width of flower head obtained from the plants cultivated in the field of TNIHS were measured to characterize both erect- and prostrate-types of growth form (Fig. 5). Among the individuals, TN-3 showed the largest values in both the length and width of flower head (2.87 mm and 1.68 mm, respectively). On the other hand, the shortest head length was found in TSY-3 (1.49 mm) and the narrowest head width was in WK-1 (0.92 mm). In erect-type, the length and width of flower head were mostly constant from 1.49 to 2.27 mm (C.V. = 10.1%) and from 0.92 to 2.00 mm (C.V. =9.2%), respectively. Contrary to this, in prostrate-type, larger variation was observed in flower head size than that in erect-type, ranging from 1.76 to 2.87 mm (C.V. = 20.3%) in length and from 1.17 to 1.68 mm (C. V. = 17.3%) in width. However, no statistical difference ($P < 0.05$, t-test) was observed in length and width of flower head between erect- and prostrate-types. The flower head size was neither associated with latitude of their location of collection nor with flowering date. In the length/width ratio of flower head, statistical difference between erect- and prostrate-types was not found (1.58 ± 0.18 and 1.49 ± 0.13), respectively.

The length and width of flower head of herbarium specimens deposited in TNS in each flowering stage are shown in Fig. 6. At Stage 1, the length and width of flower head in prostrate-type were significantly larger than those in erect-type ($P < 0.05$, t-test). On the other hand, at Stage 2, larger length of flower head was found in prostrate-type than in erect-type, while statistical difference in width of flower head was not observed between erect- and prostrate-types. Thereafter, at Stage 3 and 4, there was no significant difference between erect- and prostrate-types in both length and width of flower head.



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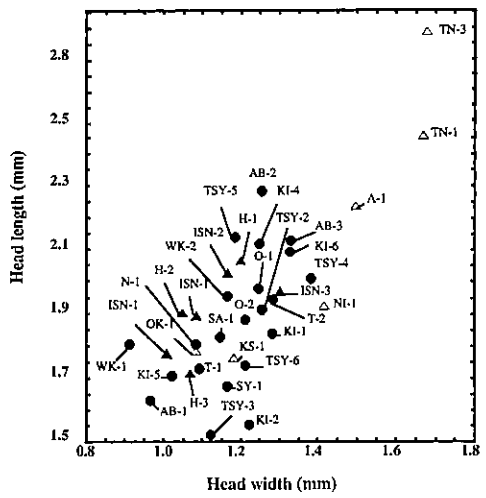
● : Erect-type collected at riverbed ; ▲ : Erect-type collected at coastline ; △ : Prostrate-type collected at coastline.
* and ns indicate significant correlation between flowering date and latitude of collecting location and nonsignificant, respectively ($P<0.05$, correlation coefficient).
Abbreviations are shown in Table 1.



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Abbreviations are shown in Table 1.

The flowering date in the prostrate-type had close relationship to latitude of their location of collection ($P<0.05$, $r=-0.913$), where individuals collected from higher latitude flowered earlier than those from lower latitude (Fig. 4). On the other hand, in the erect-type, no such relationship was observed between the flowering date and the latitude of their location of collection ($P<0.05$, $r=-0.304$) (Fig. 4). No significant difference in flowering date between erect- and prostrate-types was observed in this experiment.

Flower head size

The length and width of flower head obtained from the plants cultivated in the field of TNIHS were measured to characterize both erect- and prostrate-types of growth form (Fig. 5). Among the individuals, TN-3 showed the largest values in both the length and width of flower head (2.87 mm and 1.68 mm, respectively). On the other hand, the shortest head length was found in TSY-3 (1.49 mm) and the narrowest head width was in WK-1 (0.92 mm). In erect-type, the length and width of flower head were mostly constant from 1.49 to 2.27 mm (C.V. = 10.1%) and from 0.92 to 2.00 mm (C.V. =9.2%), respectively. Contrary to this, in prostrate-type, larger variation was observed in flower head size than that in erect-type, ranging from 1.76 to 2.87 mm (C.V. = 20.3%) in length and from 1.17 to 1.68 mm (C. V. = 17.3%) in width. However, no statistical difference ($P < 0.05$, t-test) was observed in length and width of flower head between erect- and prostrate-types. The flower head size was neither associated with latitude of their location of collection nor with flowering date. In the length/width ratio of flower head, statistical difference between erect- and prostrate-types was not found (1.58 ± 0.18 and 1.49 ± 0.13), respectively.

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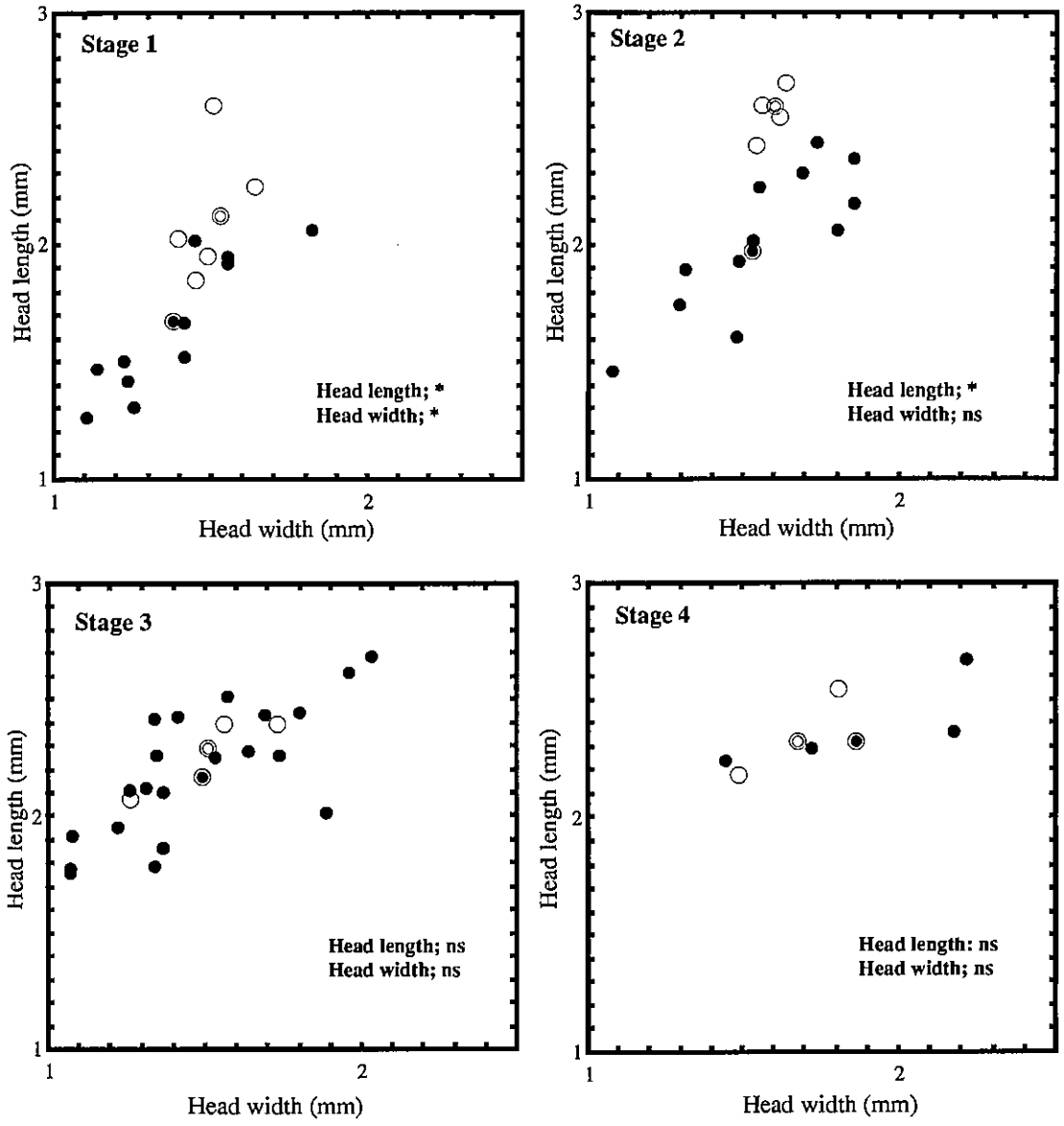

Fig. 6. Flower head size of herbarium specimens of *Artemisia capillaris* deposited in the Herbarium, Department of Botany, National Science Museum, Ibaraki, Japan.
● : Erect-type ; ○ : Prostrate type ; ◉ : Average of erect-type ; ◎ : Average of prostrate-type.
Stage 1 : Involucral bracts begin opening and ray flowers release ; Stage 2 : Ray flowers extend, disk flowers bloom and pollen diffuse ; Stage 3 : Disk flowers fully bloom and their stigmas elongate ; Stage 4 : Corollas drop, seeds in disk flowers mature and involucral bract remain.
* and ns indicates significantly different between erect- and prostrate-types and nonsignificant, respectively (P <0.05, t-test).

Table 3. Length / width ratio of flower head of *Artemisia capillaris* in herbarium specimens deposited in the Herbarium, Department of Botany, National Science Museum, Ibaraki, Japan

Growth form / Flowering stage[1)]	Stage 1	Stage 2	Stage 3	Stage 4
Erect-type	1.20±0.11 (n=11)	1.30±0.12 (n=12)	1.51±0.19 (n=21)	1.29±0.20 (n=4)
Prostrate-type	1.40±0.17 (n=5)	1.60±0.04 (n=4)	1.52±0.14 (n=3)	1.44±0.23 (n=2)
	*	*	ns	ns

1) For details, see Fig. 6.
* and ns indicate significantly different between erect- and prostrate-types and nonsignificant, respectively ($P < 0.05$, t-test).

The flower head sizes in both erect- and prostrate-types were not associated with latitude of each localities. The length/width ratio in both erect- and prostrate-types at each stage are shown in Table 3. At Stage 1 and 2, the length/width ratio of prostrate-type was significantly larger ($P < 0.05$, t-test) than that in erect-type, while such significant difference was absent at Stage 3 and 4.

Contents of CAP and DME in flower head

Contents of CAP and DME in flower head of both erect- and prostrate-types cultivated in the field of TNIHS are shown in Fig. 7 and 8. The highest contents of CAP was found in TSY-1 (1.32 %DW) and the lowest was in A-1 (trace), whereas DME content was highest in KI-2 (2.64 %DW) and the lowest in TN-3 (0.20 %DW). Our results showed that the individual having higher content of CAP not necessarily had higher content of DME in their flower head (Fig. 7).

The content of DME in erect-type (0.65 to 2.64 %DW) was significantly higher ($P < 0.05$, t-test) than that in prostrate-type (0.20 to 0.69 %DW) (Fig. 8). The content of CAP in erect-type (0.26 to 1.32 %DW) was higher than that in prostrate-type (no detect to 0.80 %DW), however, its difference was not statistically significant. Larger coefficient of variations of CAP and DME contents were found in the prostrate-type (CAP, 95.7 %; DME, 43.2%) than those in erect-type (CAP,

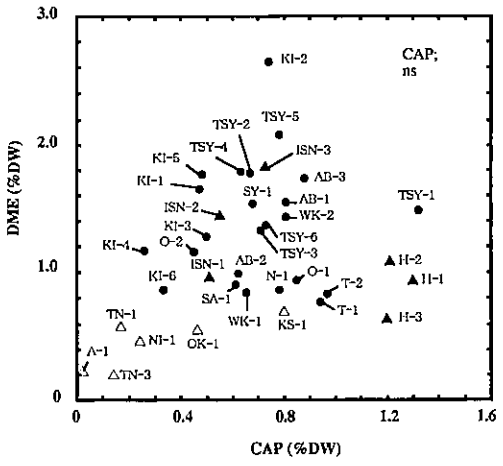

Fig. 7. The contents of capillarisin (CAP) and 6,7-dimethylesculetin (DME) in flower head of thirty six individuals of *Artemisia capillaris* collected at sixteen different locations in Japan.
● : Erect-type collected at rivervbed ; ▲ : Erect-type collected at coastline ; △ : Prostrate-type collected at coastline.
* and ns indicate significantly different between erect- and prostrate-types and nonsignificant, respectively ($P<0.05$, t-test). Abbreviations are shown in Table 1.

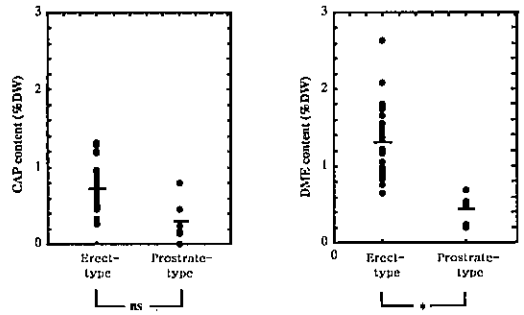

Fig. 8. The contents of capillarisin (CAP) and 6,7-dimethylesculetin (DME) in flower head of erect- and prostrate-types of *Artemisia capillaris* at sixteen different locations in Japan.
Bar indicates average of each type.
* and ns indicate significantly different from each other and nonsignificant, ($P<0.05$, t-test).

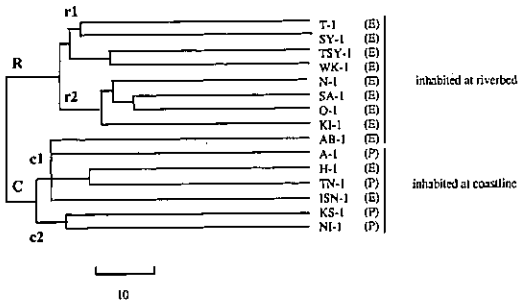

Fig. 9. Dendrogrum for classification of *Artemisia capillaris* collected at fifteen different locations in Japan. The presence or absence of amplified DNA fragment was treated as a binary character.
A dendrogram was constructed on the basis of euclidian distance by using the unweighted pair group method with arithmetic means (UPGMA).
(E) : Erect-type ; (P) : Prostrate-type. Abbreviations are shown in Table 1.

35.4 %; DME, 34.6%), and especially the DME content in prostrate-type showed remarkably large variations among the individuals. In both erect- and prostrate-types, the contents of CAP and DME were neither correlated to latitude of their location of collection nor to flower head size.

Although ISN and H accessions were collected at coastline (Table 1) but showed erect growth form (Table 2), their range of CAP content was from 0.51 to 0.73 %DW (ISN accession) and from 1.19 to 1.20 %DW (H accession), and their range of DME content was from 0.97 to 1.81 %DW (ISN accession) and from 0.65 to 0.96 %DW (H accession), respectively (Fig. 7). Thus, CAP and DME contents in these six individuals were very similar to those in erect-type collected at riverbed.

Highly sensitive random amplified polymorphic DNA methods by digoxigenin labeling (DIG-RAPD)

Fifteen individuals were screened for DIG-RAPD markers using single synthetic arbitrary sequence primer in a PCR-based DNA amplification procedure. Most primers produced banding patterns that were easily scored, and amplified DNA bands were clearly distinct and polymorphic. The seven primers resulted in a total 199 DNA fragments among fifteen individuals. Results of cluster analysis of DIG-RAPD band patterns are shown in Fig. 9.

The cluster was clearly divided into two clusters i.e. R cluster contained all erect-type accessions collected at riverbed, and it was further divided into two (r 1 and r 2) subclusters. Although eight erect-types collected from riverbed built up C-cluster, genetic relationship of inter- and intra-subclusters among eight individuals were not related with latitude of their location of collection, flowering date and flower head size. All prostrate-type accessions (collected at coastline), AB-1 (collected at riverbed and showed erect growth form) and H-1 and ISN-1 (collected at coastline but showed erect-growth form) belonged to C-cluster. C-cluster was also divided into two (c 1 and c 2) subclusters, and AB-1, H-1 and ISN-1 belonged to the same c 1 subcluster. Since the dendrograme of erect-type did not form a clear cluster, large genetic variations among erect-type individuals were thought to have occurred.

In this experiment, results of DIG-RAPD analysis clearly divided fifteen individuals into two groups according to the individuals collected from riverbed or coastline except AB-1. However, genetic relationships did not correlate with growth form, flowering date and flower head size.

Discussion

Environmental conditions in the habitat affect the morphological and physiological characters of plants and occasionally differentiate the plants into different ecotype. Tominaga et al. (1989 a) reported differences of morphological characters and pollen fertility in inland and foredune populations of *Imperata cylindrica* in Kii Peninsula, Wakayama, Japan. Their results showed that populations inhabited in inland have significantly longer plant length than those in foredune, and almost all foredune populations were male sterile, accordingly seed set percentage of inland populations was much higher than that of foredune ones. Matsumura (1967) found differences in seeds size, germination conditions and heading date between low- and up-land types in *Alopecurus aequalis*. In both types of this species, their morphological characters were not altered by reciprocal transplanting into low- and up-land field conditions, and those characters were considered to be fixed.

In *Artemisia capillaris* collected from eighteen locations in Japan in this experiment, two types of growth form were confirmed, and all nine accessions collected at riverbed were erect-type and those collected at coastline were prostrate-type except for two accessions (ISN and H accessions). This obvious difference in growth form of the plants sustained over a few years of successive cultivation in the field of TNHIS. This result well agreed with previous observation by Okanishi et al. (1974) who suggested that the growth form of prostrate-type plant collected at coastline remained even after three years of cultivation. In addition to these, the herbarium specimens of erect-type deposited in TNS were collected at various habitats, i.e. riverbed, disturbed habitats along roadway and mountain trail, and near beach, while all those of prostrate -type were collected at only coastline and near beach. This prostrate-type character in *A. capillaris* very resemble to that in beach plant (Aston and Bradshow 1966; Tominaga et al. 1989 b), and is considered to be adapted to sea breeze and movement of sea sand. In contrast, erect-type grown at the riverbed could be advantageous to competing with other species. Difference in growth form of genus *Artemisia* is well described in *A. annua* by Paniego et al. (1993), where this species grown in Europe and America exhibited great variation in size and shape, ranges from small, almost prostrate to tall, erect. Thus, it may be assumed that such difference of environmental conditions in location as riverbed or coastline could influence not only the growth form but also the physiological and other morphological characters in *A. capillaris*. Both growth forms may be the consequence of acclimating process of the plant to environmental conditions of locations, and be fixed as genetic character.

Imperata cylindrica (Tominaga et al. 1989 a), *Plantago asiatica* (Yamanishi and Fukunaga 1983) and *Arabis serrata* (Oyama 1993) widely distributed in Japan from north to south are very diverse in flowering habit having close relationship with environmental conditions of habitat. In this experiment, in prostrate-type of *A. capillaris*, flowering date was significantly related with latitude of their location of collection. From this results, it may be assumed that photoperiod affects flower differentiation of *A. capillaris*. Ferreia and Janick (1995) reported that *A. annua* is short-day plant with a critical photoperiod of 13.5 h. Therefore, our result could suggest that the prostrate-type of growth form is short-day plant. Contrary to this, in erect-type, such trend was not found. This may be because erect-type accessions collected from more narrow latitude (from 36° 44′ N to 33° 52′ N) than those of prostrate-type accessions (from 40° 57′ N to 30° 32′ N). We do not have more information whether erect-type accessions were short-day plants or not. Thus, for clarifying the photoperiodism of erect-type of growth form, we need to collect this type from more locations with wide range of latitude.

The width of flower head was shown to be an important character in the botanical description of genus *Artemisia* and was often used to identify genus *Artemisia* as well as other plants belonging to family Compositae (Kitamura 1940; Ohwi 1992). Kitamura (1940) reported that flower head size of prostrate-type was a little larger than that of erect-type. However, in this experiment, the flower head size of plants collected in the field of TNIHS had no significant difference between both growth forms. This tendency was the same as in the report of Okanishi et al. (1974). On the other hand, as see in Fig. 6, the flower head size in herbarium specimens deposited in TNS tended to vary with flowering stage. Before Stage 3, length and width of prostrate-type were significantly larger than those of erect-type, but after this stage such statistical difference disappeared.

Kitamura (1940) pointed out that the shape of flower head in prostrate-type was ovate, while that in erect-type was globose. However, as resulted from observation of herbarium specimens deposited in TNS, the length/width ratio of flower head, which reflects the shape of it, changed in process of flowering stage and significant difference of length/width ratio between erect- and prostrate-types was found at Stage 1 and 2, while after Stage 3, this difference between erect- and prostrate-types was not observed. This ratio of erect-type clearly increased from Stage 2 to Stage 3 and the shape of flower

head in erect-type changed from globose to ovate due to elongation of involucral bracts at late flowering stage, while that of prostrate-type remained ovate during flowering stage (Table 3). Our results revealed that discrimination between erect- and prostrate-types by shape of flower head could be possible only at Stage 1 and 2 of flowering, while it was difficult at Stage 3 and 4. In flower head of plants collected in the field of TNIHS, no significant difference of length/width ratio between erect- and prostrate-types may result because the flowering stage of both types proceeded to Stage 3.

Clear difference in contents of CAP and DME was observed between erect- and prostrate-types, and the average of CAP and DME contents were obviously higher in erect-type than in prostrate-type. As far as contents of CAP and DME, both types have quite different chemical character and are characterized by their contents. The results of our experiment revealed that *A. capillaris* inhabited in Japan is characterized by growth form, the shape and size of flower head at early flowering stage and the contents of CAP and DME, accordingly each type could be regarded as an ecotype.

Cluster analysis based on RAPD markers has become an important tool for determination of plant species (Dubouzet et al. 1998; Yamagishi et al. 1998) and geographic variations (Watanabe et al. 1999) and confirmation of pedigree relationship of cultivars (Lu et al. 1996). However, some cases are reported where discrimination of cultivars or subspecies was not clearly performed by this method (Sosinski and Douches 1996; Nakamura et al. 1997). Nakamura et al. (1997) reported that individuals of typical *Solidago virgaurea* ssp. *asiatica* and ssp. *leiocarpa* based on morphology were not distinguished by RAPD analysis. In this experiment, we applied DIG-RAPD analysis to examine whether the genetic differentiation is detected between erect- and prostrate-types. The result was divided into two groups according to the individuals collected from riverbed or coastline except AB-1, while it could not be clearly distinguished by the growth form. Although we do not have any information to clearly postulate this result, this result may raise the possibility that seedlings or seeds of erect-type at riverbed of inland moved to coastline with riversand by man-made disturbance, and then physiologically adapted to environmental conditions, or caused introgression with indigenous prostrate-type at coastline by some way. Further molecular analysis in detail is considered to be needed.

In this experiment, no intermediate plant in growth form between erect- and prostrate-types was observed within the limit of our collected samples. If hybridization between these two types of growth forms is possible, hybrid plants could be found around the river mouth. In order to shed light on mode of inheritance in growth form, we are searching naturally hybrid plants as well as undertaking hybridization program to make hybrid plants of erect- and prostrate-types by artificial crossing and by studying their molecular analysis.

Acknowledgments

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Appendix

Collection localities, collector and number of herbarium specimens deposited in the Herbarium, Department of Botany, National Science Museum, Tsukuba in Japan.

Erect-type

Tokyo Pref.: Shinjuku (Okuyama, 19580; TNS–258030) ; Sekido, Hachioji-shi (Ohkawa, TNS–105081) ; Nakagawara, Hachioji-shi (Ohkawa, TNS–410642; TNS-410643; TNS–436056) ; Fuchu-shi (Ohkawa, TNS–403622). **Kanagawa Pref.**: Sagami (Sakurai, TNS–11894). **Nagano Pref.**: Ohsika-mura, Shimo-ina-gun (Matsumura,738) ; Nishi-senga-taki, Karuizawa-cho, Kita-saku-gun (Kawamoto, TNS–504542) ; Oiwake, Karuizawa-cho, Kita-saku-gun (Ohkawa, TNS–431249; TNS–431250) ; Takebuchi, Matsumoto-shi (Yokouchi, TNS–394769) ; Sakai, Fujimi-cho, Suwa-gun (Takei, TNS–184487) ; Higashi-chikuma-gun (Koidzumi, TNS–176076). **Yamanashi Pref.**: Kajika-zawa-cho, Minami-koma-gun (Asakawa, 47). **Shizuoka Pref.**: Ogasa-cho, Ogasa-gun (Hashimoto, TNS–43809) ; Asagiri Plateau, Fujinomiya-shi (Ukegawa *et al.* 68) ; Kami-ide, Fujinomiya-shi (Saito, 6025) ; Hirano, Shizuoka-shi (Konta, 6323). **Kyoto Pref.**: Kono, Ujitawara-cho, Tsuzuki-gun (Tsugaru&Takahashi, 23900) ; Hozu-cho, Kameoka-shi (Tsugaru & Takahashi, 15623). **Osaka Pref.**: Matsugahara, Hirakata-shi (Tsuchiya 839) ; Yao-shi (Kodama, 8448) ; Chihaya-akasaka-mura, Minami-kawachi-gun (Murata, 20023). **Hyogo Pref.**: Matogata, Himeji-shi (Kitamura, TNS-264136) ; Wada, San-nan-cho, Hikami-gun (Hosomi, TNS–57993). **Wakayama Pref.**: Tanabe-shi (Ui, TNS–25954). **Tottori Pref.**: Fukube-mura, Iwami-gun (Murata,72401). **Yamaguchi Pref.**: Nishinohama, Hagi-shi (Nikai, TNS–45808) ; Hagi-shi (Nikai, TNS–259988) ; Ogori-cho, Yoshiki-gun (Nikai, TNS–45810) ; Atoh-cho, Abu-gun (n.s., 9551). **Kagawa Pref.**: Itano-cho, Itano-gun (Nikai, TNS–45809) ; Kokubunji-cho, Ayauta-gun (Toyoshima, TNS-143252) ; Mitoyo-gun (Takahashi, 1189). **Fukuoka Pref.**: Tsuiki-cho, Tskujyo-gun (Igami, 6191). **Kumamoto Pref.**: Uto-shi (Yamashiro,25958). **Miyazaki Pref.**: Kadokawa-cho, Higashi-Usuki-gun (Nagasawa, 105151). **Kagoshima Pref.**: Kanoya-shi (Okuyama & Ustumi, 15205; 15206). **Okinawa Pref.**: Funaura, Taketomi-cho, Yaeyama-gun (Koyama *et al.*, 288) ; Kabira, Ishigaki-shi (Yamamoto, TNS–344078) ; Taketomi-shima, Ishigaki-shi (Ito, TNS–60094) ; Miyako-jima, Hirara-shi (Amano, 6062). Okinawa (Kawarada, TNS–15088)

Prostrate-type

Aomori Pref.: Okidate, Aomori-shi (Sakurai, TNS-11896). **Fukushima Pref.**: Matsukawa-ura, Souma-shi (Nemoto, TNS-31809). **Kanagawa Pref.**: Arai, Yokosuka-shi (Saito, TNS-153334). **Aichi Pref.**: Konakayama, Atsumi-cho, Atsumi-gun (Furuse, TNS-215702). **Hyogo Pref.**: Akashi-shi (Sono, TNS-15520). **Ishikawa Pref.**: Kanaiwa, Kanazawa-shi (n.s., TNS-223523). **Shimane Pref.**: Takobi, Shimane-cho, Yatsuka-gun (Okuyama & Utsumi, 11472; 11473). **Kagawa Pref.**: Iyo-shi (Nagasawa, TNS-53114). **Fukuoka Pref.**: Higashi-ku,Fukuoka-shi (Nagata, 718). **Saga Pref.**: Ohtomo, Naruko-cho, Higashi-matsuura-gun (Baba, 12). **Nagasaki Pref.**: Kazusa-cho, Minami-takaki-gun (Yokoo, TNS-64653); Nomozaki-cho, Nishi-sonogi-gun (Thoyama, TNS-108135); Izuhara-cho, Shimo-agata-gun (Koyama, 2725). **Miyazaki Pref.**: Mimitsu, Tsuno-cho, Koyu-gun (n.s., TNS-302979). **Kagoshima Pref.**: Nishino-omote-shi (Ohuchiyama, TNS-117724); Makurazaki, Bonotsu-cho, Kawanabe-gun (Ito, 895). **Okinawa Pref.**: Kuzukube,Miyako-gun (Tachitu, 1246).

南基泰[1]・ミア　モハマド　ワヒドザマン[1]・酒井英二[1,6]・西孝三郎[1]・佐竹元吉[2]・近藤誠三[3]・岡賢治[3]・田部井豊[4]・番（古賀）保徳[5]・萱野暁明[5]・柴田敏郎[1]：カワラヨモギの「ほふく型」と「直立型」の開花日，頭花サイズ，頭花中の利胆成分含量及び分子レベルの相違について

カワラヨモギは，河岸と海岸に自生し，直立型とほふく型の草姿があることが指摘されている。しかし，これまでに両草姿型についての詳細な比較検討はされてこなかった。そこで，日本国内の18地点（河岸：9地点，海岸：9地点）より植物体もしくは種子を導入し，両草姿型の開花日，頭花サイズ，利胆成分であるcapillarisinと6,7-dimethylesculetinの頭花中の含量及びRAPD法による分子レベルでの相違について検討した。更に，国立科学博物館植物部門所蔵のさく葉標本（62点）の草姿，頭花サイズの調査結果も併せて，両草姿型の各形質の相違について検討した。

河岸採集個体は，すべて直立型であった。一方，海岸採集個体は，ほとんどがほふく型であったが，一部直立型の個体が確認された。直立型とほふく型では，開花の早晩性には差がなかった。頭花サイズは，総苞片が展開し始めた段階では，ほふく型の方が直立型よりも長さ，幅共に有意に長かったが，筒状花が開花し，花粉粒が飛散するころには，両草姿型共にほぼ同じサイズとなり，区別ができなくなった。頭花中の6,7-dimethylesculetin含量は，直立型の方がほふく型よりも有意に高かった。一方，capillarisin含量も直立型の方が高含量であったが，両草姿型間で有意な差は認められなかった。直立型及びほふく型共に，筑波薬用植物栽培試験場で保存栽培した結果，それぞれの形質は維持され続けた。従って，両草姿の各形質は，遺伝的な要因によるものであり，直立型とほふく型は，それぞれ異なる生態型（エコタイプ）と考えられた。そこで，両草姿型間の分子レベルでの相違を検出するため，開花が認められた15個体より全DNAを抽出し，ジゴキシゲニンをラベリングした10 merのプライマーによって増幅したPCR産物の電気泳動パターンより，クラスター分析を行った。その結果，河岸採集個体と海岸採集個体との2グループにほぼ分かれたが，草姿，頭花中の利胆成分含量によるグループ分けとは完全には一致しなかった。

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