植物研究雑誌
J. Jpn. Bot.
70: 253–259 (1995)

Multivariate Analysis of Needle Size and Its Anatomical Traits of *Pinus* Subgenus Haploxylon (Soft Pines) on Mt. Tateyama, Toyama Prefecture, Japan

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(Received on December 19, 1994)

Through the previous studies by this author, four types of Pinus subgenus Haploxylon, *P. pumila*, TENGU-type and OIWAKE-type of *P. hakkodensis*, and *P. parviflora*, were distinguished by seed size and life form, were recognized. In this study, multivariate analysis of their needle size and 15 morphological traits were carried out to examine the variation among the populations. The results of discriminant analysis indicated that the populations are grouped into two subclusters, consisting of *P. pumila* and TENGU-type, and *P. parviflora* and OIWAKE-type, respectively. This result well agrees with the results of seed morphology and cones and ovuliferous scales reported previously. Consequently, TENGU and OIWAKE types may be recognized as extreme forms of *P. pumila* and *P. parviflora*, respectively.

Hayashi (1960) and Oota et al. (1983) reported that three species of soft pines belonging to *Pinus* subgenus Haploxylon, *Pinus pumila* (Pallas) Regel, *P. hakkodensis* Makino and *P. parviflora* Sieb. et Zucc., were growing in Mt. Tateyama located in Toyama prefecture.

The name of *P. hakkodensis* was published by Makino and Nemoto (1931) with a short description as an intermediate species between *P. pumila* and *P. pentaphylla*, having ascendent stems. Since then, it has been considered as a hybrid between *P. pumila* and *P. parviflora* (Hayashi 1960, Kitamura and Murata 1979, Satake 1989), or as a variety of *P. pentaphylla* Mayr (Iwata and Kusaka 1954).

Sato (1993a) recognized four types of soft pine populations in Mt. Tateyama based on the variation analysis of seed size and life form. The first type was identified as *P. pumila*. TENGU-type and OIWAKE-type were identified as *P. hakkodensis* because both of their intermediate features between *P. pumila* and *P. parviflora* as described by Makino and Nemoto (1931). The fourth type was identified as *P. parviflora*. Allometric analysis of seed scale morphology revealed difference between *P. pumila* and *P. parviflora*. TENGU-type similar to *P. pumila*, is distinct from OIWAKE-type, which is similar to *P. parviflora* (Sato 1993b).

Koehne (1893), Hayata and Satake (1929), Doi and Morikawa (1929), Ishii (1938) and Kausik and Bhattacharya (1977) suggested that anatomical traits of needles were useful to classify *Pinus*. On the basis of the number and arrangement of resin ducts, the Siberian *P. pumila* were more similar to the Hokkaido plants than to the Honshu plants (Sato 1993c). Therefore, to obtain the helpful evidences to understand the taxonomical and phylogenetical relationships among four types of soft pines in Mt. Tateyama, morphological and anatomical studies on needles were under-taken.

Sampling procedure and Method

Eleven populations of soft pines in Mt. Tateyama including four populations of *P. pumila*, two of TENGU-type, three of OIWAKE-type and two of *P. parviflora* (Table 1, Fig. 1) were chosen for this study. Twenty trees, growing more than 2 m away from each other, were sampled per population. One branchlet was taken from a sunny crown of each tree because shade needles are smaller (Shimakura, 1934). Five one-year-old fascicles were removed from a branchlet. The site of measurement for each characteristic was shown in Table 2 and Fig. 2. For the study of external morphology, one needle was removed at random from each fascicle, and about 7 needles per tree were measured for their length (NL). Number of serrations on the abaxial edge of needles (NSE) was counted on a central 5 mm section of needle. For the study of anatomical characters, one needle per fascicle was removed and stocked in 80% alcohol. The central part of the needle was sectioned and examined

Table 1. Populations studied and their sampling size

Localities	Altitude (m)	Sample size	Sampling date	Type
1 MURODO-A	2450	22	24. Sep. 1990	*P. pumila*
2 MURODO-B	2400	35	2. Sep. 1990	*P. pumila*
3 OOTANI	2310	20	2. Sep. 1990	*P. pumila*
4 TENGU	2300	21	2. Sep. 1990	TENGU-type
5 KAGAMIISHI	2250	22	2. Sep. 1990	TENGU-type
6 MIMATSU	2080	21	2. Sep. 1990	*P. pumila*
7 OIWAKE	1900	20	24. Sep. 1990	OIWAKE-type
8 NANAMAGARI	1700	21	24. Sep. 1990	OIWAKE-type
9 DAIKANDAI	1450	20	24. Sep. 1990	OIWAKE-type
10 DAIKANDAI	1450	21	24. Sep. 1990	*P. parviflora*
11 SHIMONOKODAIRA	1380	23	24. Sep. 1990	*P. parviflora*

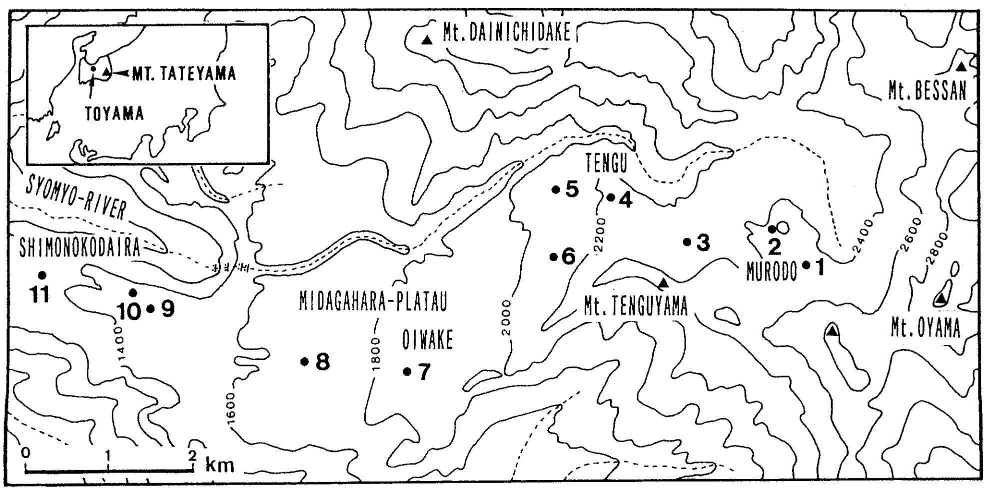

Fig. 1. Eleven populations of soft pines observed in Mt. Tateyama.

under the light microscope for 15 characters shown in Fig. 2.

Discriminant analysis has been used to clarify the relationship among species complex and to study geographical variation (Smouse and Saylor 1973a, b, Piedra 1983, McCune 1988). This analysis has also proved to be useful in characterizing differences between predefined groups, particularly where hybridization may be involved (Mayer and Mesler 1993). Thus the discriminant analysis was carried out on the

Table 2. Needle characters used in multivariate analysis

Code of character	Character	Units or coding
<Gross form of needle>		
NL	Needle length	mm
NSE	Number of serration on a central 5mm section of needle	n
<Anatomical character in cross section>		
NR	Number of resin duct	
FI	Frequency of idioblast in mesophyll	%
NST	Number of stomata per one lateral side	n
NH	Number of hypoderm layer per abaxial side	n
NSX	Number of sclerenchyma cell in xylem side of transfusion tissue	n
NSS	Number of sclerenchyma cell in sieb side of transfusion tissue	n
LA	Length of abaxial side	μm
LL	Length of lateral side	μm
DE	Diameter of endoderm circle	μm
DR	Diameter of the largest resin duct	μm
DI	Distance between external resin ducts	μm
RE	Ratio DE/LL	ratio
RR	Ratio DR/LL	ratio
RD	Ratio DI/DE	ratio
RDL	Ratio DI/LR	ratio

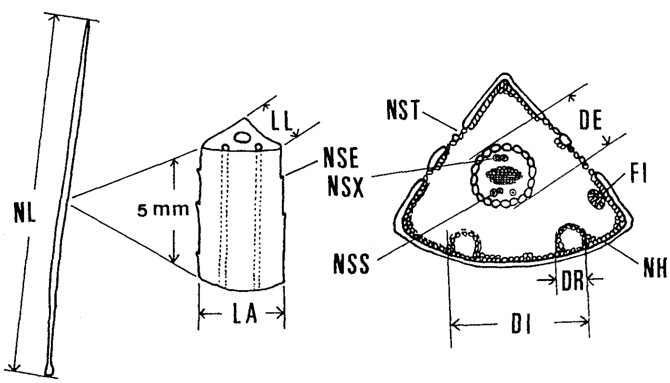

Fig. 2. Schematic drawing of needle and its cross section, indicating the sites of measurement for each characteristic.

Table 3. Mean values of needle characters within populations and types

	Characters*																
	NL	NSE	NR	FI	NST	NH	NSX	NSS	LL	LA	DE	DR	DI	RE	RR	RD	RDL
<Population>																	
1 MURODO-A	43	1.8	1.6	0	4.5	2.0	0	0	758	904	289	109	362	0.38	0.14	1.18	0.37
2 MURODO-B	50	2.1	1.7	0	4.0	2.5	0	0	740	882	301	102	359	0.41	0.14	1.17	0.39
3 OOTANI	45	1.8	1.5	14	4.1	1.3	0	0.1	751	897	286	108	361	0.38	0.14	1.15	0.37
4 TENGU	59	2.5	1.8	41	4.9	1.9	0	0.2	906	1102	368	115	449	0.41	0.13	1.16	0.38
5 KAGAMIISHI	58	1.8	1.8	61	5.7	1.9	0	0.2	945	1164	370	114	445	0.39	0.12	1.19	0.38
6 MIMATSU	57	1.4	1.6	38	4.7	1.8	0	0	820	968	320	110	394	0.39	0.14	1.16	0.39
7 OIWAKE	47	2.9	1.8	0	4.3	1.1	1.7	5.6	850	1054	354	90	419	0.42	0.11	1.18	0.40
8 NANAMAGARI	50	2.7	1.8	0	4.6	1.1	2.1	6.9	893	1041	357	74	409	0.40	0.08	1.14	0.39
9 DAIKANDAI	51	2.4	1.8	0	5.0	1.2	1.8	6.7	915	1105	362	84	434	0.40	0.09	1.19	0.38
10 DAIKANDAI	52	2.8	2.1	0	5.5	1.0	4.2	5.4	931	1095	365	66	475	0.39	0.07	1.30	0.43
11 SHIMONOKODAIRA	45	2.5	1.7	0	3.5	1.0	3.5	5.0	722	892	268	67	450	0.37	0.09	1.69	0.50
<Type>																	
P. pumila	49	1.8	1.6	13	4.3	1.9	0.0	0.0	767	913	299	107	369	0.39	0.14	1.16	0.38
TENGU-type	58	2.1	1.8	51	5.3	1.9	0.0	0.2	926	1133	369	114	447	0.40	0.12	1.17	0.38
OIWAKE-type	49	2.7	1.8	0	4.6	1.1	1.9	6.4	886	1067	358	83	421	0.41	0.09	1.17	0.39
P. parviflora	49	2.7	1.9	0	4.5	1.0	3.9	5.2	826	993	316	67	463	0.38	0.08	1.49	0.46

*Abbreviation of characters should be referred toTable 2.

needle characters of each population.

Results and Discussion

The mean value of external morphological data and anatomical data in each population are shown in Table 3. The needle length (NL) of three adjacent populations, TENGU, KAGAMIISHI and MIMATSU, is more than 55 mm, being longer than those of other populations.

The numbers of serration (NSE) of *P. pumila* and TENGU-type were fewer than those of the other types in average.

The numbers of resin ducts (NR) per needle in most trees of *P. parviflora* were more than two. Several trees of this type have three resin ducts, two external and one middle or lateral. Most of the resin ducts were located on the abaxial side of needle. In *P. pumila* and TENGU-type the needles which have three resin ducts were not observed except one sample from MIMATSU population. *Pinus parviflora* found to have two external and one middle or lateral resin ducts positions in the needle.

The idioblasts in mesophyll (FI) were found in the TENGU-type and the TENGU, KAGAMIISHI, OOTANI and MIMATSU populations of *P. pumila*, which were located closely. Ishii (1941) recognized a new type of soft pine with the idioblast in mesophyll on Mt. Zao.

The mean numbers of stomata per lateral face (NST) of TENGU-type were more than those of other types. The mean numbers of cell layers in the hypoderm (NH) of *P. pumila* and TENGU-type were more than one, while other types mostly consisted of a single cell layer.

The sclerites in the transfusion tissue (NSX and NSS) were found in the samples of OIWAKE-type and *P. parviflora*. Very few sclerites were observed in a few samples of TENGU-type and only one sclerite was found in a sample of OOTANI-population. In OIWAKE-type, the mean number of sclerites in the adaxial side (NSX) was fewer than that number in the abaxial side (NSS). The mean number of sclerites in adaxial side (NSX) of OIWAKE-type was fewer than that of *P. parviflora*. Doi and Morikawa (1929) and Ishii (1941) recognized that the presence of sclerite in the transfusion tissue was one of distinct characteristics of *P. parviflora*. According to their criteria, OIWAKE-type should belong to *P. parviflora*.

The mean values of LL, LA, DE and DR in TENGU-type were significantly longer than those of other types. The mean values of the RR in TENGU-type and OIWAKE-type were intermediate between *P. parviflora* and *P. pumila*, The mean values of the RD and RDL in *P. parviflora* were bigger than those of other three types, but the differences in this ratio were insignificant.

Discriminant analysis was carried out on the 14 leaf characters of each population, excluding DI, RD and RDL.

A discriminant function was computed from *P. pumila* and *P. parviflora* samples and the residual covariance matrices. A combination character index was then obtained by scoring each tree according to the following equation:

$$\text{Discriminant score} = \sum_{i=1}^{14} a_i Y_i$$

where 'a' is the character weights derived from the discriminant analysis and 'Y' is the observed values. Discriminant functions are shown in Table 4.

The mean values of the discriminant score and its 95% confidence limits of each population are shown in Fig. 3. It is evident that there is a clear disjunction between the *P. pumila* and TENGU-type populations and the *P. parviflora* and OIWAKE-type populations. In the former cluster, there were no significant differences between the *P. pumila* populations (No. 1, 2, 3, 6) and TENGU-type populations (No. 4, 5). In the latter cluster, the mean scores of OIWAKE-type populations exhibited a clinal change corresponding

Table 4. Discriminant functions and correlations of each variables to each discriminant scores

Characters	Discriminant function	r
NL	–0.00218	0.126
NSE	–0.00594	0.321
NR	–0.02550	0.145
FI	0.08368	0.389
NST	0.00161	0.000
NH	0.05386	0.760***
NSX	–0.02554	0.885***
NSS	–0.02128	0.917***
LL	–0.00015	0.138
LA	0.00004	0.118
DE	0.00069	0.063
DR	0.00133	0.834***
RE	1.10759	0.126
RR	–0.19253	0.811***
Residual	–0.10059	
MAHALANOBIS D-SQUA	53.9	
F-VALUE F(14, 62)	96.1	

***Correlation significant at the 0.1% probability level

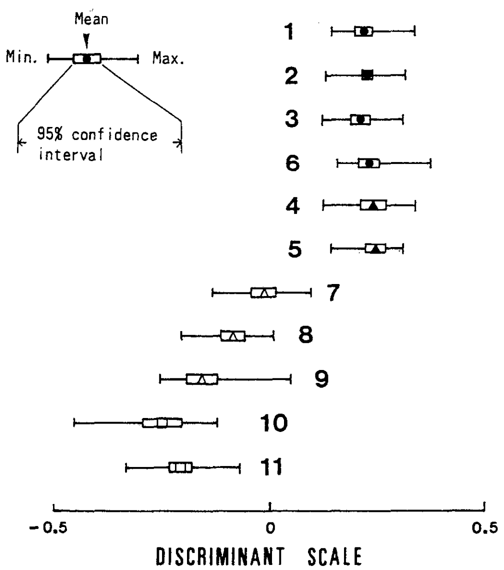

Fig. 3. Mean values of discriminant score and its 95% confidence limits of each population. 1~3, 6: *Pinus pumila*, 4, 5: TENGU-type, 7~9: OIWAKE-type, 10, 11: *P. parviflora*.

to the elevation of the locations and the smallest mean score had no significant difference from those of *P.*

Table 5. Result of discriminant analysis

Type	Discriminant score Mean	Std.
P. pumila	0.22	0.01
TENGU-type	0.24	0.00
OIWAKE-type	–0.09	0.06
P. parviflora	–0.23	0.01

parviflora populations. Thus, OIWAKE-type may not be recognized as distinct in the needle anatomy. This result will agree with the results obtained in the previous studies of seed morphology (Sato 1993a) and cones and scales (Sato 1993b). Consequently, TENGU-type and OIWAKE type, previously recognized as *P. hokkaidensis* were apparently different, could from each other and may be recognized as extreme forms of *P. pumila* and *P. parviflora*, respectively.

Coefficients of correlations between the discriminant scores and each variable are shown in Table 4. The discriminant scores are distinctly high in the five variables, NH, NSX, NSS, DR and RR. That indicates that these five characters are suitable to distinguish *P. pumila* from *P. parviflora*, and are also important to understand relationships in *P. parviflora* group and the related taxa.

After de Ferré (1966) presented that *P. pumila* is most closely allied to the *P. parviflora* group, Mirov (1967) and Critchfield (1986) took a similar view. Mirov (1967) proposed "*parviflora* complex" including Chinese and South East Asian white pines (*P. kwangtungensis*, *P. wangi*, *P. morrisonicola* etc.) and he assumed that through *P. pumila* the "parviflora complex" is possibly connected with *P. sibirica*.

By using other analyses, review of the relationship between *P. pumila* and *P. parviflora* will be needed after this.

Anonymous reviewers are much appreciated for their invaluable suggestions. This study was supported in part by a Grant-in-Aid (No. 05917010) for scientific research from the Ministry of Education, Science and Culture.

Literature Cited

Critchfield W. B. 1986. Hybridization and Classification of the white pines (*Pinus* section *strobus*). Taxon **35**(4): 647–656.

Doi T. and Morikawa K. 1929. An anatomical study of leaves of the genus *Pinus*. J. Dep. Agr. Kyushu Imp. Univ. **2**: 149–198.

de Ferré Y. 1966. Validité de l'espéce *Pinus pumila* et affinités systématiques. Bull. Soc. Hist. Nat. Toulouse **102**: 351–356.

Hayashi Y. 1960. Taxonomical and Phytogeographical Study of Japanese Conifers. pp.152–159. Norin Shuppan, Tokyo (in Japanese).

Hayata B. and Satake Y. 1929. Contributions to the knowledge of systematic anatomy of some Japanese plants. Bot. Mag. Tokyo **43**: 73–106 (in Japanese).

Ishii S. 1938. On the various forms of *Pinus pumila* distinguished by the structure of leaves with special reference to their distribution. J. Jap. For. Soc. **20**: 1-16 (in Japanese).

——— 1941. On the various forms of *P. pumila* and other northern Japanese soft pines with special reference to their distribution. J. Jap. For. Soc. **23**: 47–55 (in Japanese).

Iwata T. and Kusaka M. 1954. Coniferae Japonicae Illustratae. pp. 214. Sangyotosho, Tokyo (in Japanese).

Kausik S. B. and Bhattacharya S. S. 1977. Comparative foliar anatomy of selected gymnosperms: Leaf structure in relation to leaf form in Coniferales and Taxales. Phytomorphology **27**(2): 146–160.

Kitamura S. and Murata G. 1978. Coloured Illustrations of Woody Plants of Japan II. p.248. Hoikusha, Osaka (in Japanese).

Koehne E. 1893. Deutsche Dendrologie. pp.28–33. Stuttgart.

Makino T. and Nemoto K. 1931. Flora of Japan. 2nd ed. p.148. Shunyodo Shoten, Tokyo (in Japanese).

Mayer M. and Mesler M. 1993. Morphometric evidence of hybrid swarms in mixed populations of *Polystichum munitum* and *P. imbricans* (Dryopteridaceae). Systematic Botany **18**(2): 248–260.

McCune B. 1988. Ecological diversity in North American pines. Amer. J. Bot. **75**(3): 353–368.

Mirov N. T. 1967. The genus *Pinus*. 602 pp. Ronald Press, New York.

Oota H., Shoji T. and Nagai S. 1983. Flora of Toyama, Japan. pp. 151–152. Koubundo, Toyama (in Japanese).

Piedra T. E. 1983. Geographic variation in needles, cones and seeds of *Pinus tecunumanii* in Guatemala. Silvae Genetica **33**: 72–79.

Satake Y. 1989. Pinaceae. In: Satake Y. et al. (ed.) Wild Flowers of Japan, Woody Plants **I**: 5–8. Heibonsha, Tokyo (in Japanese).

Sato T. 1993a. Seed morphology of *Pinus* subgenus Haploxylon on Mt. Tateyama, Toyama Prefecture, Japan. J. Phytogeogr. & Taxon. **41**: 7–13 (in Japanese).

——— 1993b. Morphological variation of cones and ovuliferous scales in *Pinus* subgenus Haploxylon on Mt. Tateyama, Toyama Pref., Japan. J. Jap. Bot. **68**(5): 277–288 (in Japanese).

——— 1993c. Leaf anatomy of Siberian and Japanese plants of *Pinus pumila* Regel. Ann. Rep. Bot. Gard. Kanazawa Univ. **16**: 19–24.

Shimakura T. 1934. Structure of sunny and shade leaves on some conifer. Plants and Animals **2**: 1043–1052 (in Japanese).

Smouse P. E. and Saylor L. C. 1973a. Studies of the *Pinus rigida-serotina* complex I. A study of geographic variation. Ann. Missouri Bot. Gard. **60**: 174–191.

——— 1973b. Studies of the *Pinus rigida-serotina* complex II. Natural hybridization among the *Pinus rigida-serotina* complex, *P. taeda* and *P. echinata*. Ann. Missouri Bot. Gard. **60**: 192–203.

佐藤　卓：立山産ゴヨウマツ類の葉のサイズと解剖学的形質の多変量解析

富山県立山にはハイマツ，キタゴヨウ，天狗型ハッコウダゴヨウ，追分型ハッコウダゴヨウの4タイプのゴヨウマツが報告されている．そこでこれらのゴヨウマツ類の類縁関係を明らかにするため，針葉のサイズと解剖学的形質の多変量解析を行った．その結果，ハイマツと天狗型ハッコウダゴヨウ及びキタゴヨウと追分型ハッコウダゴヨウという2つの明瞭に異なる亜群が識別された．天狗型ハッコウダゴヨウはハイマツと全く区別できなかった．また，追分型ハッコウダゴヨウはキタゴヨウに連続することが明らかになった．この傾向は本著者によるゴヨウマツ類の種子形態や球果などについての変異の分析結果とよく一致するものである．従って，立山でハッコウダゴヨウと呼んだ天狗型と追分型はそれぞれハイマツとキタゴヨウの極端型ではないかと考えられる．