Medico-Botanical Studies of Ephedra Plants from the Himalayan Region: [Part 2] Geographical Variation in the Anatomical Characters of Herbal Stems,and the Estimation of the Original Locality of Tibetan Crude Drugs "TSHE" and "BALU"

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Medico-Botanical Studies of *Ephedra* Plants from the Himalayan Region Part II. Geographical Variation in the Anatomical Characters of Herbal Stems, and the Estimation of the Original Locality of Tibetan Crude Drugs "TSHE" and "BALU"

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Geographical variations of the inner structures of the herbal stems of *Ephedra gerardiana* Wall. ex Stapf and *E. pachyclada* Boiss. of the family Ephedraceae related to longitudinal and altitudinal changes were studied. In *E. gerardiana*, longitudinal geoclines on the diameter of the herbal stem and the number of subepidermal fiber bundles, the plant in the western areas tended to have bigger stems in diameter and more subepidermal fiber bundles than those in eastern areas were recognized. In *E. pachyclada* altitudinal geoclines that the plant growing at higher ground tended to have more fibers in a subepidermal fiber bundle and more pith fibers were recognized. Moreover, local variations were recognized in both species; in *E. gerardiana*, most of the plants from Mid. Western Nepal had more than 40 cortical fiber bundles and less than 50 pith fibers, and in *E. pachyclada* those from Mid. Western Nepal had smaller stems in diameter and more cortical fiber bundles, etc. These results enables to estimate original localities of Tibetan crude drugs of *Ephedra*. (Continued from J. Jpn. Bot. **71**(6), 323–332, 1996)

Plants of *Ephedra* (Ephedraceae) are well known medicinal resources, including an alkaloid ephedrine. In the previous paper (Mikage and Kondo 1996) we reported that *E. gerardiana* Wall. ex Stapf and *E. pachyclada* Boiss. growing in Himalayan region were distinguished anatomically by the difference of the number of subepidermal fiber bundles in the cross section of herbal stems. It was also revealed that many anatomical characters showed wide intraspecific variations.

As to the variations of outer morphology and alkaloid contents in relation to different habitat of *E. pachyclada*, Mikage et al. (1987) reported that the plant growing at dried places or the upper part of a slope had rather shorter internodes than those growing at wet places or the bottom of a slope, and the former contains more alkaloids than the latter in amount.

In the Chinese traditional medicine, it has been said that the original locality of medicinal plant is an important information to evaluate the medicinal value of it. Though it is generally difficult to identify the original locality of crude drugs circulated in the markets, it may become possible if local characteristics are found in the inner structures of the drugs.

On the other hand, there have been a few anatomical reports on the geoclinal variation in the size of the vessel elements in the genera *Symplocos* (Oever et al. 1981) and *Ilex* (Bass 1973), and in the family Oleaceae (Bass et al. 1988), correlated to latitude or altitude.

In this report, to study geoclinal variations

in the anatomical characters of *E. gerardiana* and *E. pachyclada*, each anatomical character of the internodes in considering the collecting places of the experimental materials such as longitude and altitude is examined. Based on the results, the original locality of Tibetan crude drugs TSHE and BALU, the whole plant of *Ephedra* spp. (Mikage and Kondo 1996) are estimated.

Materials and Methods

In addition to the materials used in the previous paper (Mikage et al. 1996), four plants are newly examined.

Ephedra gerardiana – Mid. Western Nepal, Karnali Zone, Dolpa Dist., between Hanke and Sumduwa [G57: Watanabe et al. (WT) 9507108 ♀], between Sumduwa and Ringmo [G58: WT9507004 ♀]. *E. pachyclada* – Mid. Western Nepal, Karnali Zone, Dolpa Distr., between Dunai and Juphal Airport [P62: WT9507112 ♀. P63: WT9507116 ♀].

The cross sections of three internodes of herbal stems arbitralily chosen from each plant were observed and the average value of the three was obtained in each of the anatomical characteristic such as the diameter of stem, number of subepidermal fiber bundles, maximum number of fibers in a subepidermal fiber bundle, number of cortical fiber bundles, and number of fibers in the peripheral part of pith. And the correlations between these average values and the longitude and altitude of the collecting points of experimental materials were examined. Besides, 10 herbal stems were examined to know the variations in one plant, and five plants to know the variations in a colony.

For the convenience of studying local variations, the areas where the samples of *E. gerardiana* were collected for this study are divided into five by administrative divisions; i.e., the Areas I (Mid. Western Nepal), II (Western Nepal), III (Central Nepal), IV (Eastern Nepal), and V (Sikkim and Bhutan). So as the Area II where the samples of *E. pachyclada* were collected is altitudinally divided into five zones; the Alt. zones i (between Larjung and Tukuche, 2460 to 2620m above sea level), ii (between Tukuche and Jomsom, 2600 to 2690m), iii (between Jomsom and Kagbeni, 2700 to 2815m), iv (between Kagbeni and Jharkot, 2840 to 3340m), and v (between Jharkot and Muktinath, 3380 to 3460m).

Results

The variation range of measured values of each anatomical character and their coefficient of variation (C.V.) calculated in one plant, in a colony, in each Area, in each Alt. zone, and in materials, respectively, are shown in Tables 1 on *E. gerardiana* and 2 on *E. pachyclada*.

I. Anatomical variation relating to longitudinal change

1. *Ephedra gerardiana* (Table 1, Fig. 1)

1) Diameter of herbal stem (Fig. 1-1)

The diameter of herbal stem is rather stable in one plant and in a colony; the C.V. is 6.4% and 1.8%, respectively. The western samples tend to have bigger stems than the eastern ones in diameter. Though the significant difference ($p<0.05$) is recognized between average values in the Area I and Area V, the variation is geographically successive from west to east: the correlation coefficient (C.C.) is –0.521.

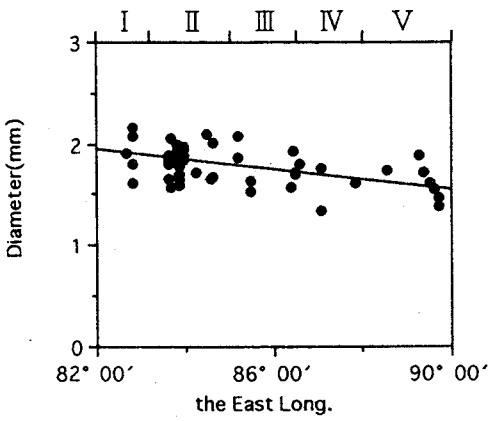
2) Number of subepidermal fiber bundles (Fig. 1-2)

Number of subepidermal fiber bundles is rather variable in one plant, the C.V. is 11.0%. Generally to say, the western plant has more subepidermal fiber bundles than eastern one: C.C. is –0.437. Though the significant difference ($p<0.05$) is recognized between the average values in the Area I and Area V, the variation is geographically successive.

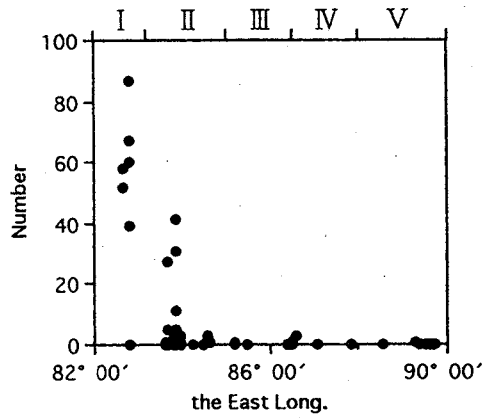
Besides, in the Area II, a local variation is recognized; i.e., plants growing along the

Table 1. Anatomical characteristics of herbal stems of *Ephedra gerardiana*, summed up in different orders

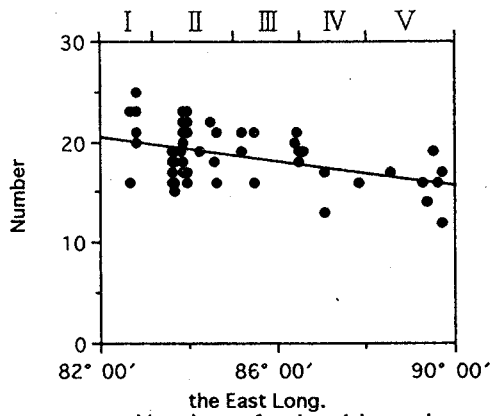
		Single plant	Single colony	Area I	Area II	Area III	Area IV	Area V	All materials
Diameter of herbal stem (mm)	MIN.–MAX.	1.74–2.09	1.81–1.88	1.62–2.16	1.57–2.09	1.53–2.07	1.35–1.80	1.38–1.88	1.35–2.16
	(MEAN ± S.D.)	(1.89 ± 0.12)	(1.84 ± 0.03)	(1.93 ± 0.18)	(1.84 ± 0.13)	(1.75 ± 0.18)	(1.63 ± 0.20)	(1.62 ± 0.17)	(1.80 ± 0.18)
	C.V.	6.4	1.8	9.5	7.2	10.5	12.5	10.6	9.9
Number of subepidermal fiber bundles	MIN.–MAX.	15–22	16–19	16–25	15–23	16–21	13–19	12–19	12–25
	(MEAN ± S.D.)	(19 ± 2.06)	(17 ± 1.06)	(21 ± 2.69)	(19 ± 2.35)	(19 ± 1.69)	(16 ± 2.54)	(16 ± 2.38)	(19 ± 2.72)
	C.V.	11.0	6.1	12.7	12.6	8.7	15.9	15.1	14.7
Maximum number of fibers in a subepidermal fiber bundle	MIN.–MAX.	25–37	25–33	29–40	19–41	24–31	20–28	25–55	19–55
	(MEAN ± S.D.)	(32 ± 3.50)	(31 ± 3.42)	(32 ± 4.00)	(29 ± 5.43)	(28 ± 2.42)	(24 ± 3.39)	(39 ± 10.94)	(30 ± 6.83)
	C.V.	10.9	11.2	12.4	18.6	8.8	14.1	27.8	22.6
Number of cortical fiber bundles	MIN.–MAX.	0–1	0–1	0–87	0–41	0–1	0–3	0–1	0–87
	(MEAN ± S.D.)	(0 ± 0.42)	(0 ± 0.33)	(52 ± 27.14)	(4 ± 9.72)	(0 ± 0.39)	(1 ± 1.33)	(0 ± 0.25)	(9 ± 19.81)
	C.V.	210.8	100	52.3	221.2	155.3	200.0	264.6	225.6
Number of pith fibers	MIN.–MAX.	41–99	63–77	3–45	7–211	40–186	108–166	66–262	3–262
	(MEAN ± S.D.)	(69 ± 18.02)	(69 ± 5.59)	(21 ± 14.17)	(89 ± 53.36)	(127 ± 48.32)	(132 ± 25.94)	(131 ± 64.75)	(94 ± 58.53)
	C.V.	26.0	8.1	67.5	60.2	38.1	19.7	49.4	62.4



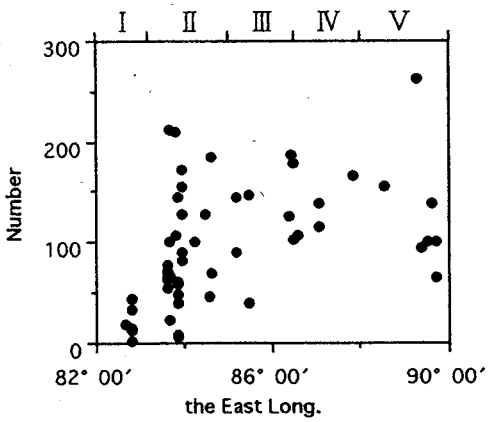
1.Diameter of herbal stem (mm)



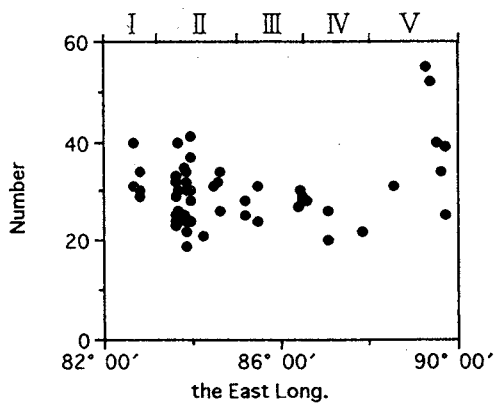
4. Number of cortical fiber bundles



2. Number of subepidermal fiber bundles



5.Number of pith fibers



3. Maximum number of fibers in a subepidermal fiber bundle

Fig. 1. Geographical variation in anatomical characteristics of herbal stems of *Ephedra gerardiana*. I: Area I. II: Area II. III: Area III. IV: Area IV. V: Area V.

Kaligandaki river between Tukuche, Yak Kharka, and Alubari have exceptionally few fiber bundles, 15 to 19.

3) Maximum number of fibers in a subepidermal fiber bundle (Fig. 1-3)

Maximum number of fibers in a subepidermal fiber bundle is variable to some extent. Though the variation is successive, characteristic local variations are recognized; i.e., the plants from the Area V show bigger values, while those from III and IV show smaller values.

4) Number of cortical fiber bundles (Fig. 1-4)

Since most of the plants have few cortical fibers, the C.V. calculated become enormously big. Therefore, as to this character, the standard deviation (S.D.) is examined instead.

Though a large intraspecific variation is recognized on this character, it is rather stable in one plant and in a colony: S.D. are 0.42 and 0.33, respectively, while S.D. in all the materials is 19.81. Plants from the Areas III, IV and V are usually have no cortical fiber bundle, and some plants from the Area II have 1~11 bundles, while most of the plants from the Area I have more than 40 bundles. A significant difference ($p<0.01$) is recognized between the average value in the Area I and that in the other four Areas each. It can be said that the plant from the Area I is characterized by having many cortical fibers, mostly more than 40.

5) Number of pith fibers (Fig. 1-5)

Number of pith fibers shows wide intraspecific variation. Plants from the Area I have usually less than 50 fibers, while the plants from the Areas IV and V have more than 50. A significant difference ($p<0.01$) are recognized between the average value in the Area I and that in the other four Areas each.

2. *Ephedra pachyclada*

Of 64 samples of this species, only four are from the Area I, and the rest from the Area II. Though the samples from the Area I are few in number, they tend to have smaller stem in diameter, more cortical fiber bundles, and less pith fibers (Fig. 2).

II. Anatomical variation relating to altitudinal change

Variation relating to altitudinal change was examined on *E. pachyclada* collected from the Area II (Table 2, Fig. 3).

1) Diameter of herbal stems (Fig. 2-1)

Variation in the diameter of herbal stems is rather small in one plant and in a colony. A significant difference is not recognized between the average values in each zones.

2) Number of subepidermal fiber bundles (Fig. 2-2)

The plants having many subepidermal fiber bundles are rare in higher altitudes. There is a significant difference ($p<0.05$) between the average values of the plant in the Alt. zones iv or v and zone ii.

3) Maximum number of fibers in a subepidermal fiber bundle (Fig. 2-3)

Plants growing at higher places tend to have more fibers in a subepidermal fiber bundle than those at lower places, C.C. is –0.353. The observed value is less than 25 in the plants from the Alt. zones i, ii, and iii, while some plants from the zones iv and v have more than 25.

4) Number of cortical fiber bundles (Fig. 2-4)

Number of cortical fiber bundles widely varies in a single plant, in a colony, and in all the materials. No significant difference can be seen between the average values in the Alt. zone i to v. On the other hand, in the Area I, all the plants examined have more than 50 bundles, i.e., observed values are in the range of 54 to 68 with average to 58, while those from the Area II are in the range of 6 to 72 and the average of each Alt. zone are in the range of 26 to 29.

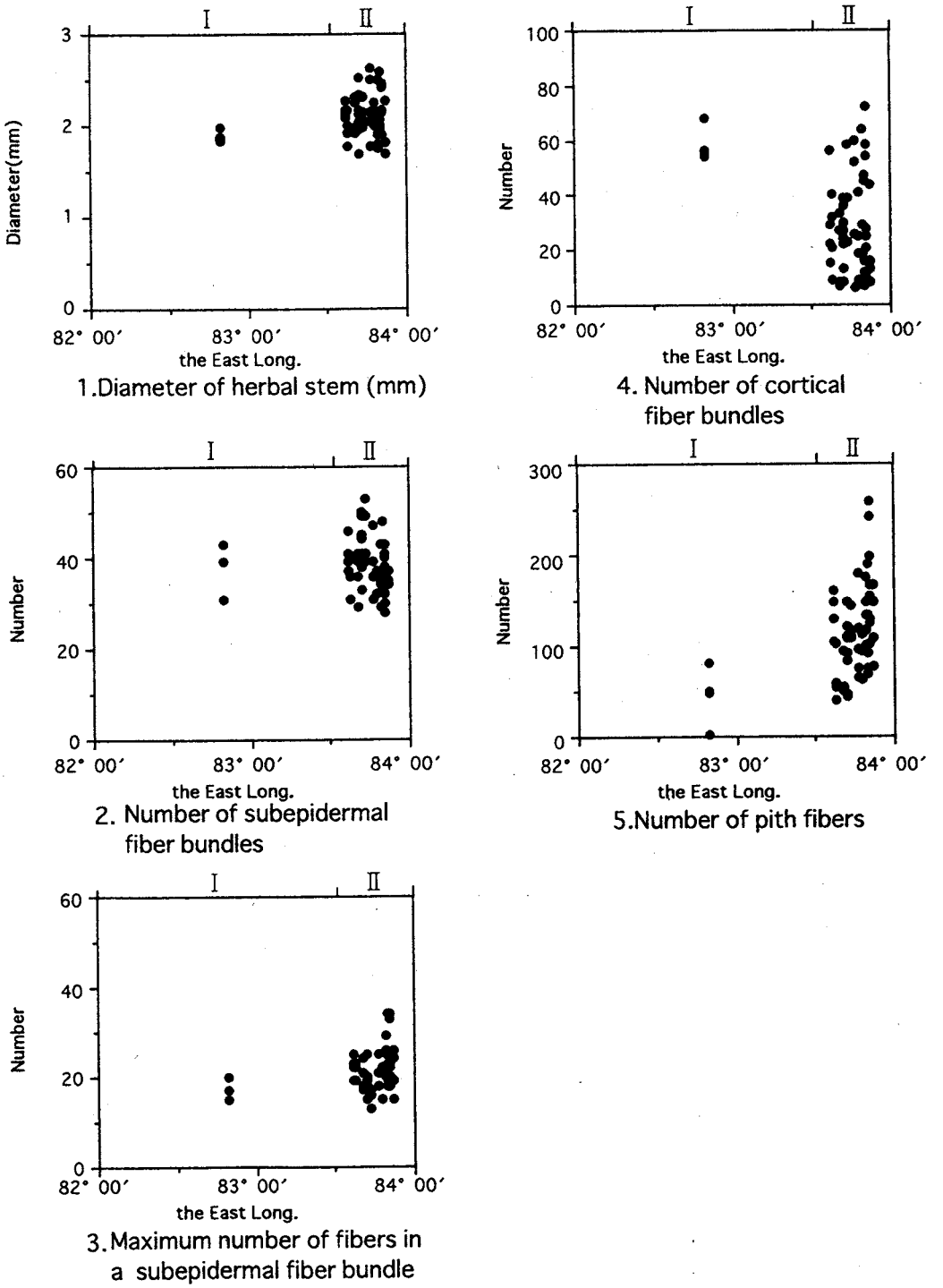


Fig. 2. Geographical variation in anatomical characteristics of herbal stems of *Ephedra pachyclada* (relating to longitudinal changes). I: Area I. II: Area II.

Table 2. Anatomical characteristics of herbal stems of *Ephedra pachyclada*, summed up in different orders

		Single plant	Single colony	Area I	Area II					All materials
					Alt. zone i	Alt. zone ii	Alt. zone iii	Alt. zone iv	Alt. zone v	
Diameter of herbal stem (mm)	MIN.–MAX.	1.82–2.26	2.17–2.53	1.83–1.97	1.77–2.28	1.68–2.53	1.77–2.62	1.76–2.58	1.68–2.45	1.68–2.62
	(MEAN ± S.D.)	(2.08 ± 0.14)	(2.34 ± 0.13)	(1.88 ± 0.06)	(2.06 ± 0.16)	(2.13 ± 0.22)	(2.18 ± 0.26)	(2.04 ± 0.24)	(2.12 ± 0.27)	(2.11 ± 0.23)
	C.V.	6.6	5.5	3.2	7.9	10.2	11.8	12.0	12.9	11.2
Number of subepidermal fiber bundles	MIN.–MAX.	28–41	44–50	31–43	31–46	29–53	31–47	29–48	28–43	28–53
	(MEAN ± S.D.)	(34 ± 4.40)	(46 ± 3.13)	(38 ± 5.26)	(38 ± 4.26)	(42 ± 7.15)	(38 ± 5.72)	(36 ± 5.22)	(36 ± 4.24)	(38 ± 5.75)
	C.V.	13.1	6.7	13.9	11.2	17.2	15.0	14.6	11.9	15.2
Maximum number of fibers in a subepidermal fiber bundle	MIN.–MAX.	17–25	15–20	15–20	19–25	13–25	15–25	18–34	15–34	13–34
	(MEAN ± S.D.)	(22 ± 2.66)	(18 ± 1.84)	(17 ± 1.99)	(22 ± 2.09)	(18 ± 2.77)	(20 ± 2.83)	(23 ± 4.43)	(23 ± 5.36)	(21 ± 4.32)
	C.V.	12.4	10.4	11.5	9.6	15.8	13.9	19.2	23.4	20.8
Number of cortical fiber bundles	MIN.–MAX.	11–38	8–36	54–68	9–56	7–58	6–60	7–64	8–72	6–72
	(MEAN ± S.D.)	(24 ± 9.86)	(21 ± 10.88)	(58 ± 6.54)	(28 ± 14.84)	(28 ± 13.69)	(27 ± 20.03)	(26 ± 19.08)	(29 ± 21.09)	(30 ± 18.54)
	C.V.	41.6	51.5	11.2	52.9	48.9	73.8	74.6	72.8	62.7
Number of pith fibers	MIN.–MAX.	59–140	83–148	2–81	40–161	44–148	63–180	68–189	78–258	2–258
	(MEAN ± S.D.)	(98 ± 25.80)	(105 ± 25.60)	(45 ± 32.51)	(100 ± 45.15)	(98 ± 32.85)	(102 ± 35.87)	(117 ± 37.87)	(152 ± 54.24)	(111 ± 47.98)
	C.V.	26.5	24.4	72.8	45.2	33.4	35.3	32.4	35.7	43.3

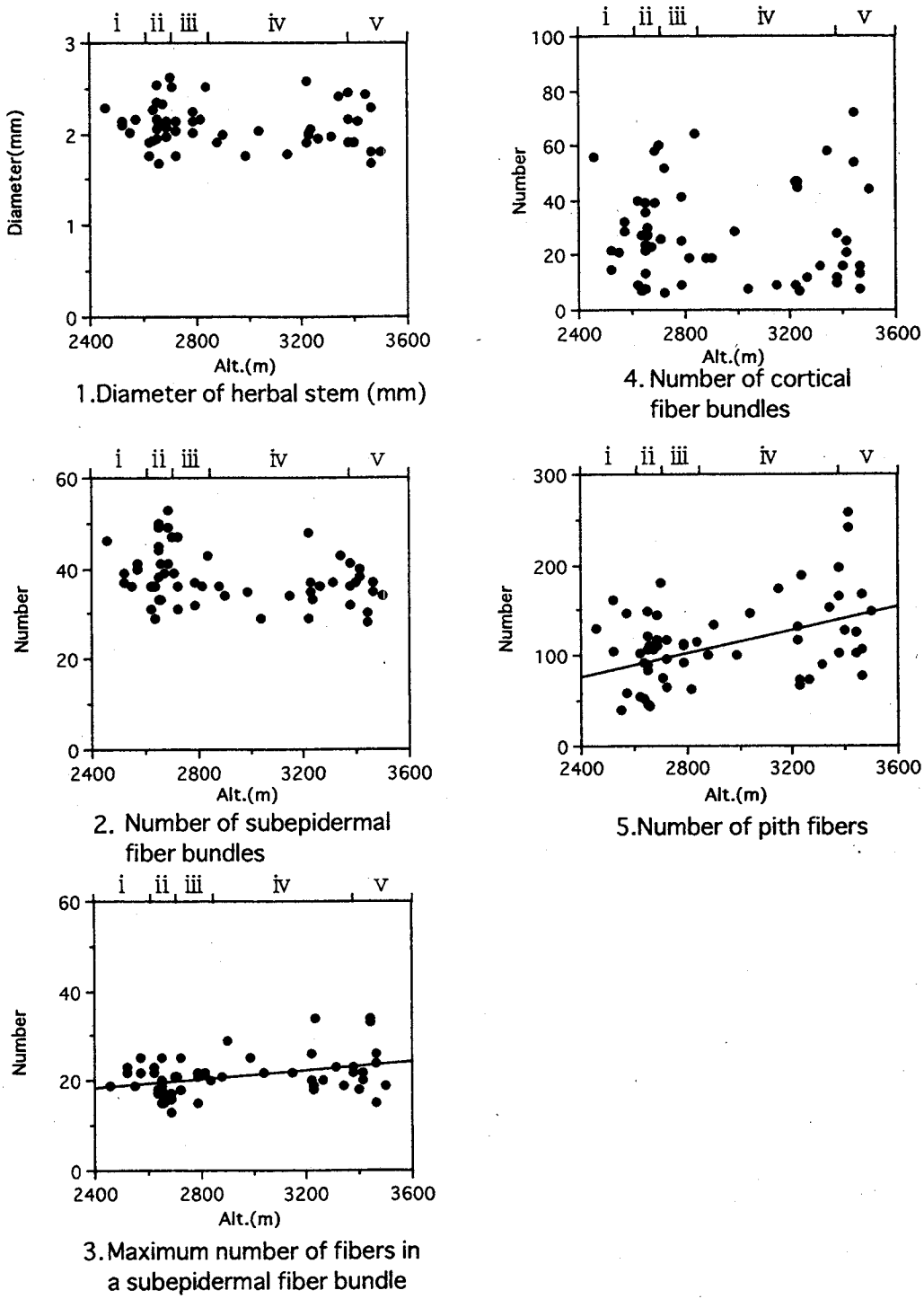


Fig. 3. Geographical variation in anatomical characteristics of herbal stems of *Ephedra pachyclada* (relating to altitudinal changes). i: Alt. zone i. ii: Alt. zone ii. iii: Alt. zone iii. iv: Alt. zone iv. v: Alt. zone v.

5) Number of pith fibers (Fig. 2-5)

Number of pith fibers is variable in one plants, in a colony, and in all the materials. Plants growing at higher places tend to have more pith fibers than those at lower places: C.C. is 0.373. A significant difference ($p<0.05$) was recognized between the average value in the Alt. zone v and that in the other four lower zones each.

III. Estimation of the original localities of Tibetan crude drugs "BALU" and "TSHE"

1) "BALU" (KANP 1305), aerial parts of *E. gerardiana*

A sample was obtained from a traditional medical hospital in Bhutan. This sample was estimated to be *E. gerardiana* collected in Bhutan or Sikkim, because it has many fibers in a subepidermal fiber bundle: the observed value of which was in the range from 36 to 49.

2) "TSHE" (KANP 2415), aerial parts of *E. gerardiana*

A sample was obtained from a Tibetan medical hospital in Kathmandu, Nepal. The characteristics having no cortical fiber, and having rather small number of pith fibers, 56–97, are well corresponded to those of *E. gerardiana* growing in the Western and Central Nepal.

3) "TSHE" (KANP 3038), aerial part of *E. pachyclada*

A sample was obtained from a Tibetan medical hospital in Jharkot, Mustang District, Dawlagiri Zone, Nepal. This sample was estimated to be *E. pachyclada* collected at higher places of Western Nepal, because of its bigger stems, having more than 20 fibers in a subepidermal fiber bundle, rather small number of cortical fibers, and many pith fibers: observed values were 2.11 to 2.39 mm in diameter, 37 to 43, 32 to 51 and 128 to 144, respectively.

Discussion

1. As the result of studying the geographical variations of the inner structures of the herbal stems of *Ephedra gerardiana* related to longitude between Mid. Western Nepal and Bhutan, a geocline that the western samples had bigger stems in diameter and more subepidermal fiber bundles was observed. Moreover, local variations were recognized; i.e., the most of the plants growing in Mid. Western Nepal had characteristically more than 40 cortical fibers, and those in Sikkim and Bhutan had more than 50 fibers in a subepidermal fiber bundle. Besides, in the variation related to altitude on the samples of *E. pachyclada*, tendencies that plants growing at higher places have less subepidermal fiber bundles, more fibers in a subepidermal fiber bundle, and more fibers in the pith, were recognized.

Through this study, it was revealed that the characteristics having been believed as the histotaxonomical diagnostics of *Ephedra* plants such as diameter of herbal stems, presence or absence of cortical fiber and the fiber in the pith, etc. (Konoshima 1945), showed wide intraspecific variations geographically. This fact suggests that a large number of samples from wide geographical range should be examined for histotaxonomical studies on the genus. The variation related to altitude and latitude is thought to be caused by the change of temperature, while the factors of the variation with longitude is not sure.

2. Stapf (1889) described one variety having smaller stems in *E. gerardiana*, var. *sikkimensis*, which was said to be growing in Bhutan, Sikkim, and Eastern Nepal. However, the description did not give the exact range of variation in the characters, and we could not distinguish the variety among the samples examined in this study. It is notable, however, that plants from Sikkim and Bhutan, tend to have less subepidermal fiber bundles, more fibers in a subepidermal fiber bundles, more fibers in the pith, and smaller diameter of herbal stems than those of other western plants, while the plants from the Mid. Western Nepal

have cortical fibers. But, the variations of these factors are geographically continuous, so the experimental materials could not be underclassified clearly by these factors.

3. As the result of making clear the details of local variations of *E. gerardiana*, TSHE used in Kathmandu and BALU in Bhutan, both are aerial parts of this species, could be estimated to have been collected its near surroundings; i.e., the former collected in the Western or Central Nepal and the latter in Bhutan or Sikkim. As for TSHE used in the Western Nepal Himalaya, aerial parts of *E. pachyclada*, it was estimated to have been collected at higher altitudes in Western Nepal.

In Chinese traditional medicine, the original locality of crude drug is thought to be one of important information for evaluating the medicinal quality. Though it has been generally considered to be difficult to tell the original locality of the crude drugs circulated in markets, the result of this study suggests that it may be possible to some extent by studying the details of local variations of inner structures of the medicinal parts of the original plants.

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御影雅幸，近藤直子：**ヒマラヤ産Ephedra属植物の研究 第2報．草質茎の内部形態的特徴の地理的変異とチベット薬物「TSHE」および「BALU」の産地**

前報で，ヒマラヤ地域に分布する*Ephedra gerardiana* Wall. ex Stapf と *E. pachyclada* Boiss. は，組織分類学的に草質茎の横切面において表皮下繊維群数の相違により明確に区別されることを報告した．一方，他の内部形態的要素をも含めて各形質は大きな種内変異を示した．そこで，本研究では各形質の変異と実験材料生育地の経度と標高との相関を検討した．その結果，*E. gerardiana*では西方の株ほど草質茎の直径が大きくなり表皮下繊維群数が多くなるという，経度の変化に伴う連続的な変異(クライン)が認められた．また，*E. pachyclada*では高地に生える株ほど表皮下繊維群の1群を構成する細胞数と髄内繊維数が多くなるという標高に伴う連続変異が認められた．緯度や標高に伴うクラインは日照時間や気温の変化などに起因することが考えられるが，経度の変化に伴うクラインに関しては如何なる要因で生じるのか現時点では不明である．またそれぞれの種で，皮層部の繊維群数や髄内繊維数などに地域的な特徴が認められた．

本研究結果から，前報で原植物を解明した本属由来のチベット薬物について，それらの産地を類推することができた．中国医学では生薬の産地は品質を評価する上で重要であるが，これまで市場品の産地を証明する手段はなかった．今回の結果は内部形態の地理的変異を精査することによりそれが可能であることを示唆している．

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