



Studies of *Ephedra* Plants in Asia. Part 4.¹⁾ Morphological differences between *Ephedra sinica* Stapf and *E. intermedia* Schrenk et C.A.Meyer, and the botanical origin of Ma-huang produced in Qinghai Province

Naoko FUSHIMI,^{a)} Lili WANG,^{a)} Shunsuke EBISUI,^{b)} Shaoqing CAI^{c)} and Masayuki MIKAGE^{*a)}

^{a)}Graduate School of Natural Science & Technology, Kanazawa University, Kakuma-machi, Kanazawa, 920-1192 Japan. ^{b)}Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, 920-1192 Japan. ^{c)}School of Pharmaceutical Sciences, Peking University, 38 Xue-yuan Road, Haidian District, Beijing, 100083 China. (Received March 24, 2008. Accepted May 28, 2008.)

Ephedra plants collected mainly in Inner Mongolia and Qinghai Province, China, were studied morphologically to clarify the specific differences between *E. sinica* Stapf, *E. intermedia* Schrenk et C.A.Meyer, and *E. distachya* L. recorded in China. The results obtained were as follows: *E. sinica* and *E. distachya* recorded in China were the same species; *E. sinica* and *E. intermedia* could be stem-anatomically distinguished from each other by examining both the number of fiber bundles in the cortex and the number of fibers in the pith into account, and the species of commercial samples of Ma-huang as well as sterile herbarium specimens that include no cones were identified by this method; All *Ephedra* plants collected in the eastern region of Qinghai Province, where *E. sinica* was reported to be distributed, were identified as *E. intermedia*; The limited plant of *E. intermedia* without cones collected in the east of Qinghai Province might possibly have been identified as *E. sinica* in the past; The plant origin of the Ma-huang produced in Qinghai Province, which is collected in the eastern regions such as Longyangxia district, was thought to be *E. intermedia*.

Key words *Ephedra sinica*, *Ephedra intermedia*, *Ephedra distachya*, morphology, anatomy, Ma-huang.

Introduction

We have carried out interdisciplinary studies on *Ephedra* plants, the ephedrine containing medicinal plant, since 1983. As one of this series of studies, we analyzed the *Ephedra* plant resources in Qinghai Province, where *Ephedrae* Herba production takes place in China. In the Flora Qinghaiica,²⁾ *Ephedra sinica* Stapf, *E. intermedia* Schrenk et C.A.Meyer, *E. equisetina* Bunge, *E. przewalskii* Stapf, *E. minuta* Florin, *E. monosperma* Gmel. ex C. A. Meyer, and *E. gerardiana* Wall. ex Stapf are described, and the former three species were prescribed as the plant origin of *Ephedrae* Herba, Ma-huang in Chinese, in both Chinese and Japanese pharmacopoeias.^{3,4)} In the Flora Qinghaiica, *E. sinica* was described as being distributed in the east and north of Qinghai Province, which is the main Ma-huang production regions in the province. As described in the Flora of China,^{5,6)} there are no *E. sinica* habitats in Qinghai Province, and we received the same information from a researcher of the Northwest Institute of Plateau Biology, CAS, Xining, Qinghai, in 2002.

In the first step of this research, we visited Qinghai Province and studied the distribution of *Ephedra* plants. The result showed that species of *E. intermedia*, *E. equisetina*, and *E. przewalskii* were found, while *E. sinica* was not. Meanwhile, *E. intermedia* was found in all the collections

site where *E. sinica* had been recorded. Morphologically, *E. sinica* and *E. intermedia* can be apparently identified by differences in the shapes of the micropylar tube of seed cones. It was also reported that both species could not be identified from each other only by the morphology of the stem without cones.⁷⁾ Indeed, we found that some plants of *E. intermedia* should be identified as *E. sinica* based on the key to the species when stems without cones were observed. From these facts described above, we thought that the plant reported as *E. sinica* in Flora Qinghaiica might possibly be *E. intermedia*.

On the other hand, *E. sinica* was denominated by Stapf⁸⁾ in 1927 on the basis of a specimen collected in Hebei Province. However, there are disagreements about this species in China currently; some reports^{5,7,9)} suggest that *E. sinica* is synonymous with *E. distachya* L. and other reports^{6,10)} maintain *E. sinica* is an independent species. In China *E. sinica* and *E. distachya* have been botanically separated mainly by the terminal cone of the former species and the axillary cone of the latter.

As described above, there are some difficulties in the identification of *E. sinica* and *E. intermedia* regarding whether *E. sinica* is an independent species or a synonym of *E. distachya*. To clarify these points and to describe the Ma-huang produced in Qinghai Province, we carried out morphological and anatomical studies on the *Ephedra* plants collected mainly in Qinghai Province and Inner Mongolia.

*To whom correspondence should be addressed. e-mail : mikage@p.kanazawa-u.ac.jp

Materials

The materials were collected from June to September 2002 in Inner Mongolia and Qinghai Province. The sample data is shown in order of, collection site, altitude, (specimen number, sex). All samples were kept in the herbarium of the Faculty of Pharmaceutical Sciences, Kanazawa University (KANP), Japan.

Ephedra sinica Stapf

(*shows the specimen having terminal cones, while no mark axillary cones)

Inner Mongolia: Yan-dian, Da-la-te Qi, E-er-duo-si Shi, 1085 m (M. Mikage *et al.*, which is abbreviated to MM in the following, 02118 ♀); He-xing-gong-xing-zheng Cun, Sai-wu-su Zhen, Xing-he Xian, Wu-lan-cha-bu Meng, 1395 m (MM 02124 ♀); Ping-di-quan Zhen, Ji-ning Shi, Wu-lan-cha-bu Meng, 1285 m (MM 02128 ♂); Xi-dong-gou Cun, San-yi-quan Da-dui, Hong-sha-ba Xian, Feng-zhen Shi, Wu-lan-cha-bu Meng, 1175 m (MM 02131 ♀*); Er-dao-he-zi Cun, Xi-lao-fu Zhen, Song-shan Qu, Chi-feng Shi, 925 m (MM 02140 ♂*, MM 02141 ♀*); San-bao-shan Cun, Xinhui Zhen, Ao-han Qi, Chi-feng Shi, 760 m (MM 02143 ♀); Bai-yin-ta-la Cun, Bai-yin-ta-la Zhen, Nai-man Qi, Tong-liao Shi, 455 m (MM 02145 ♀, MM 02146 ♂); Mu-li-tu Zhen, Tong-liao Shi, 250 m (MM 02147 ♀, MM 02149 ♂); Ga-qi-qia Zhen, Ke-er-qin-zou-yi-hou Qi, Tong-liao Shi, 300 m (MM 02150 ♀); Cheng-guan Zhen, Xing-he Xian, 1280 m (MM 02703); Ping-di-quan Zhen, Ji-ning Shi, Wu-lan-cha-bu Meng, 1340 m (MM 02704); Dong-hong-sheng Xiang, Wu-chuan Xian, 300 m (MM 02705); A-ri-si-leng-tu-su-mu-ai-li, Hang-jin Qi E-er-duo-si Shi, 1390 m (MM 02707). **Hebei Province:** Hua-shu-lin Cun, Yu-dao-kou Xiang, Wei-chang-man-zu-meng-gu-zu-zi-zhi Xian, Cheng-de Shi, 1345 m (MM 02139 ♂); Huai-an Xian, 900 m (MM 02701, MM 02702). **Shanxi Province:** San-tiao-jian Cun, Da-tong Xian, Da-tong Shi, 975 m (MM 02133 ♂*); Da-qiao Cun, Zhang-xi-he Xiang, Tian-zhen Xian, Da-tong Shi, 1000 m (MM 02135 ♀).

Ephedra intermedia Schrenk et C.A. Meyer

Qinghai Province: Qing-shui Xiang, Xun-hua Xian, 1880 m (MM 02301 ♀); Xia-tang Cun, Qing-shui Xiang, Xun-hua Xian, 1805 m (MM 02302 ♀); Bao-an Xiang, Tong-ren Xian, 2260 m (MM 02306 ♀); Mai-xiu-chang-qiao-bian, Tong-ren Xian, 2720 m (MM 02307 ♀); Sha-gou Xiang, Gui-nan Xian, 2680 m (MM 02308 ♀, MM 02309 ♀); He-dong-Xiang, Gui-de Xian, 2140 m (MM 02310, MM 02311); Ga-rang Xiang, Gui-de Xian, 2225 m (MM 02312, MM 02313 ♀); Zhan-jia Cun, Hu-zhu Xian, 2195 m (MM 02315 ♀, MM 02316 ♀); Yu-run Xiang, Le-du Xian, 1990 m (MM 02317 ♀); Hong-shui Xiang, Le-du Xian, 1820 m (MM 02318); Cha-ka Zhen, Wu-lan Xian, 3110 m (MM 02319 ♀, MM 02320 ♀). **Gansu Province:** Dang-jin-shan-kou, A-ke-sai-ha-sa-ke-zu-zi-zhi Xian, 2700 m (MM 02341).

Methods

The outer morphological characteristics were observed with the naked eye and a magnifier. For the anatomical study, transverse sections of internodes or herbal stems were observed using an optical microscope without treatment or after clarification with chloral hydrate solution. For the determination of lignified cell walls, phloroglucin solution and hydrochloric acid were added as required. Three stems with one to six secondary xylem cell layers between vascular bundles were arbitrarily chosen from each of the specimens to make the maturity of experimental stems uniform.

Results

On the taxonomy of *Ephedra sinica* Stapf and *E. distachya* L.

1) Morphological characteristics (Fig. 1)

It was reported that the important morphological difference between *E. sinica* and *E. distachya* is the difference in the position of the cones, that is, the seed cones of *E. sinica* are terminal while those of *E. distachya* are axillary.¹⁰⁾ We



Fig. 1 Plants of *Ephedra sinica* grown in the same habitat; with terminal cones (left, ♂) and with axillary cones (right, ♀). (Nan-dai-he, Qin-huang-dao, Hebei Province, China)

examined the wild community of *E. sinica* in Inner Mongolia, and found that the plant with terminal seed cones and those with axillary cones grew in the same community (Fig. 1). Therefore, we concluded that the position of seed cones is not an accurate characteristic for the identification of *E. sinica* and *E. distachya*.

2) Anatomical characteristics of the stem

The anatomical characteristics of the stems of *Ephedra* plants have been reported previously.¹⁰⁻¹⁴ It was said that the anatomical character to discriminate *E. sinica* and *E. distachya* was the difference in the number of fiber bundles in the cortex and of fibers in the pith, as those in *E. sinica* are very infrequent while those in *E. distachya* are abundant.¹⁰ Therefore, samples identified as *E. sinica* by the character of the micropylar tube collected in Inner Mongolia were examined by counting the number of fiber bundles in the cortex and fibers in the pith. The result showed that the data varied continuously and the samples examined could not be separated into two groups (Fig. 2). Moreover, no relationship between these characteristics and the position of seed cones on the stem was found. These findings support

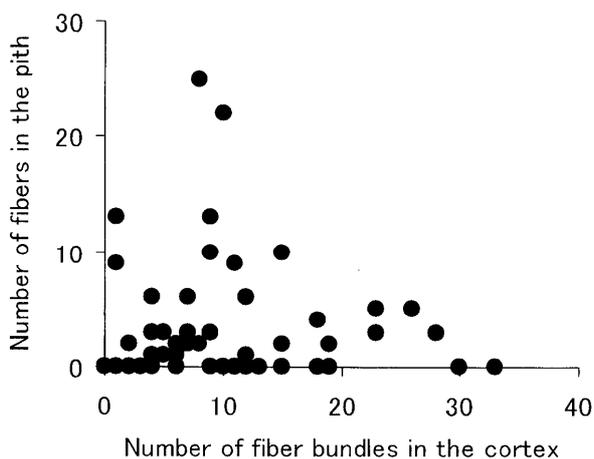


Fig. 2 Correlation between the number of fiber bundles in the cortex and number of fibers in the pith, on the samples identified as *Ephedra sinica* by the character of micropylar tube, collected in Inner Mongolia. The data varied continuously and the samples examined could not be separated into two groups.

the opinion that *E. sinica* and *E. distachya* recorded in China are the same species. So, this taxon is regarded as *E. sinica* in this study, though there is an opinion that *E. sinica* is a synonym of *E. distachya*, which grows from Europe to western Asia.

On the morphology of the stems of *Ephedra sinica* and *E. intermedia*

1) Outer morphology (Table 1)

The outer morphological characteristics of the stems of *E. sinica* and *E. intermedia* were compared. The results showed that the plant of *E. intermedia* was rather bigger than *E. sinica*; the stem was bigger in both length and diameter, and the internodes were longer, than those of *E. sinica*. The number of leaves of *E. sinica* was usually 2 and was 3 in some plants, while that of *E. intermedia* was 3 or 2. However, it was difficult to distinguish samples of the two species clearly only by the stem morphology because each measurement value of these characteristics of both species overlapped (Table 1). This result agrees well with the morphological characteristics that distinguish the two species as written in the previous papers including publications on local flora,^{2,5,6,7,15-27} and the result showed that the smaller plant in size of *E. intermedia* without cones, having 2 leaves at all nodes, might possibly be identified as *E. sinica*.

2) Inner morphology (Table 2)

i) *Ephedra sinica*

In previous reports,^{10,12,15} it was said that the shape of the cambium ring on transverse sections of herbal stems of *E. sinica* was rotund and elliptical, and that the number of fiber bundles in the cortex and of fibers in the pith was quite low. While, in this study, these characteristics of the samples analyzed varied widely; besides the typical figure as reported generally (Fig. 3-A), the stem in some specimens showed trigonal cambium ring (Fig. 3-B), or a larger number of fiber bundles existed in the cortex (Fig. 3-C) or a higher number of fibers existed in the pith (Fig. 3-D).

ii) *Ephedra intermedia*

Though the trigonal cambium ring (Fig. 3-E) was mainly recognized, rotund or elliptical examples (Fig. 3-F) were also recognized to some extent. From the results, it was found that the description in previous reports^{12,15,28} that the

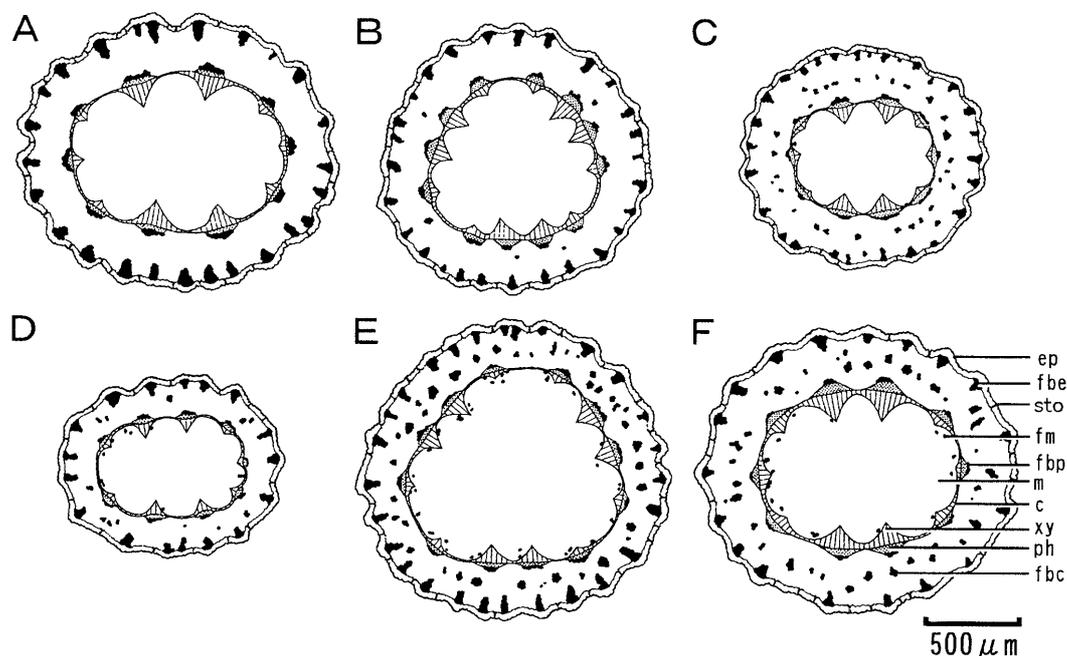
Table 1 Morphological characteristics of *Ephedra sinica* and *E. intermedia*

	<i>E. sinica</i>	<i>E. intermedia</i>
Plant height (cm)	10-40	15-60
Diameter of herbal stem (mm)	1-2	1-2
Length of internode of herbal stem (cm)	2-6	2-7
Number of leaves	2, exceptionally 3	2 or 3
Length of leaf (mm)	2.5-4	2.5-4
Degree of connection of leaf	1/3-2/3	1/3-2/3
Shape of tip of leaf	acute triangle, obtuse triangle, lanceolate	acute triangle, obtuse triangle, lanceolate
Number of seeds	2	3
Length of seed (mm)	4.5-5.5	4.5-5.5
Width of seed (mm)	2	2-2.5
Shape of micropylar tube	1-1.5mm, straight or slightly curved	long and spirally curved

Table 2 Anatomical characteristics in transverse section of herbal stems of *Ephedra sinica* and *E. intermedia*

	<i>E. sinica</i>	<i>E. intermedia</i>
Outline of transverse section	circular, elliptical, trigonal* ^{a)}	trigonal, circular*, elliptical*
Diameter of herbal stem (mm)	1.11-2.44 (1.44±0.21) ^{b)}	1.18-2.14 (1.66±0.24)
Diameter of cambium ring (mm)	0.65-1.84 (0.99±0.18)	0.82-1.65 (1.16±0.21)
Ratio of inner area of cambium ring to area of the transverse section	0.32-0.62 (0.48±0.06)	0.39-0.74 (0.49±0.06)
Existence of cuticular protuberance	exist	exist
Number of subepidermal fiber bundles	15-30 (22±3.02)	17-34 (26±3.41)
Number of fiber bundles in the cortex	1-33 (10±7.77)	26-55 (38±6.98)
Number of parenchyma cell layers in the cortex	4-7	4-7
Number of palisade cell layers	2-4	2-4
Number of large vascular bundles	2+2, 2+2+2*	2+2+2, 2+2*
Number of small vascular bundles	2+2, 3+3, 2+2+2*, 3+3+3*	2+2+2, 3+3+3, 2+2*, 3+3*
Shape of between xylem and phloem of vascular bundle	concave, flat*	flat, concave
Angle of tip of xylem	obtuse, acute	obtuse, acute
Number of fibers in pith	0, 1-25 (3±5.02)	13-107 (43±27.23)
Lignification of subepidermal fiber bundles	- ^{c)}	-
Lignification of fibers in the cortex	-, +, ++	-, +, ++
Lignification of fibers in a vascular bundle sheath	-, +, ++	-, +, ++
Lignification of fibers in the pith	-, +	-, +
Lignification of parenchyma cells in the pith	+, ++	++

^{a)} marked with *is exceptional ^{b)} (MEAN ± S.D.) ^{c)} lignification: -, not lignified, +, lignified, ++, heavily lignified

**Fig. 3** Diagram illustrating transverse sections of herbal stems of *Ephedra sinica* and *E. intermedia*.

A-D: *E. sinica*: the typical figure as generally reported (**A**); the cambium ring showed a trigonal shape (**B**); a larger number of fiber bundles existed in the cortex (**C**); a larger number of fibers existed in the pith (**D**). **E, F:** *E. intermedia*: cambium ring showed a trigonal shape (**E**), elliptical shape (**F**).

Abbreviations: c, cambium; ep, epidermis; fbe, subepidermal fiber bundle; fbc, fiber bundle in the cortex; fbp, fiber bundle of the vascular bundle sheath; fm, fiber in pith; m, pith; ph, phloem; sto, stoma; xy, xylem.

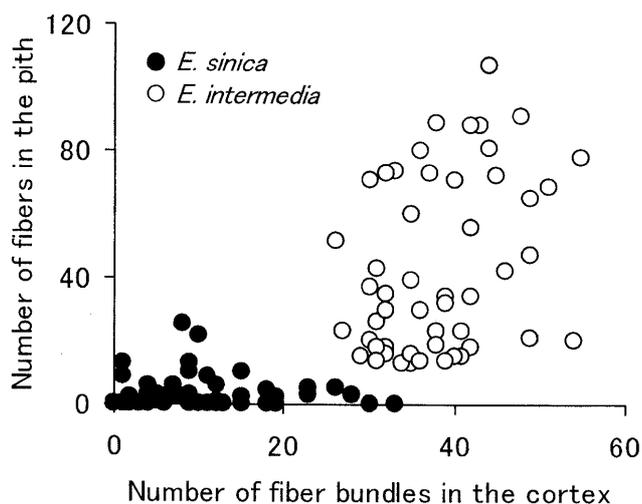


Fig. 4 Correlation between the number of fiber bundles in the cortex and number of fibers in the pith in *Ephedra sinica* and *E. intermedia*. The plot areas were separated into two groups of *E. sinica* and *E. intermedia*.

shape of the cambium ring of *E. intermedia* was trigonal was not necessarily correct.

iii) Comparative study of *Ephedra sinica* and *E. intermedia*

As described above, *E. sinica* and *E. intermedia* could not be distinguished from each other by the number of fiber bundles in the cortex or fibers in the pith because the values of both species overlapped. Then, we plotted the data of the number of fiber bundles in the cortex and of fibers in the pith on a scatter graph, and the plotted points on the graph were separated clearly into two groups, *E. sinica* and *E. intermedia* (Fig. 4); for *E. sinica*, the number of fiber bundles in the cortex was generally less than 15, and the number of fibers in the pith was less than 5 in cases of the stem having more than 16 fiber bundles in the cortex; in terms of *E. intermedia*, the number of fiber bundles in the cortex and of fibers in the pith was always more than 26 and 13, respectively. Thus, the two species could be clearly distinguished by taking into account both the number of fiber bundles in the cortex and of fibers in the pith, though it could not be done by using each character alone.

Conclusion and Discussion

1. To clarify whether or not *E. sinica* and *E. distachya* recorded in China are the same species, around 20 samples of *Ephedra* plants collected from Inner Mongolia, Hebei, Shanxi, and Gansu Provinces, where both species were said to be distributed, and were examined anatomically. Based on the numbers of fiber bundles in the cortex and of fibers in the pith, as these were found to be the best characteristics to distinguish the two species, not all the samples examined could be separated into one of the two groups. This result agreed well with the opinion that *E. sinica* and *E. distachya* recorded from Inner Mongolia are the same species. Further study is needed to determine if this species is synonymous with *E. distachya* originally recorded in Europe.

2. The morphological characters of *E. sinica* and *E.*

intermedia examined in this study showed wide variations even in one plant or in each species. In particular, in the anatomical characters in transverse sections of the stem of *E. sinica*, it was found that some stems had a trigonal cambium ring, and that the number of fiber bundles in the cortex and of fibers in the pith was more than ever reported for the species.

3. The shape of cambium ring in the cross section of the internode was correlated with the number of leaves at the lower side node; the 3-leaved internode has trigonal ring, while the 2-leaved elliptical. In addition, we speculate that the stem of larger and well-grown plants, both *E. sinica* and *E. intermedia*, tend to have three leaved internodes, though the plant of latter species is usually larger than the former.

4. By a comparative anatomical study on sufficient number of *E. sinica* and *E. intermedia* collected from Inner Mongolia and Qinghai Province, it was found that the two species could be distinguished clearly by taking into account both the numbers of fiber bundles in the cortex and of fibers in the pith. The result is useful to identify commercial samples of Ma-huang as well as sterile herbarium specimens without attached cones, which is a taxonomically important organ.

5. All *Ephedra* plants collected in the eastern region of Qinghai Province, where *E. sinica* was reported to be distributed, were identified as *E. intermedia* judging from our above-mentioned criteria. The small specimens of *E. intermedia* without attached cone might have been identified as *E. sinica* in the past. Therefore, we considered that the plant origin of the Ma-huang from Qinghai Province, which was collected in Longyangxia, *et al.*,²⁹⁾ was actually *E. intermedia*.

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