Pollen Morphology of Japanese *Oxytropis* (Leguminosae)

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The pollen morphology of the four Japanese *Oxytropis* species was examined by scanning electron microscopy. The genus was characterized by prolate tricolporate pollen grains with perforate to reticulate exine structures. *Oxytropis shokanbetsuensis*, which is endemic to Mt. Shokanbetsu, Hokkaido, northern Japan, was distinguished from the other Japanese *Oxytropis* species by having pollen grains with more fine perforate exine structures (diam. of perforations less than 0.3 μm), indistinct equatorial constrictions of the colpi and a longer polar diameter.

Key words: Pollen Morphology, Japanese *Oxytropis*

Oxytropis (Leguminosae) consists of about 300 species[1] of perennial herbs or shrubs which are found in temperate and arctic regions of the Northern Hemisphere. In Japan, five[2,3] or six[4-6] *Oxytropis* species grow in dry and stony areas of the alpine zone.

References to earlier descriptions and illustrations of pollen grains of the genus *Oxytropis* are listed by Thanikaimoni[7-11] and Tissot[12]. They conducted all pollen morphological studies on *Oxytropis* by light microscopy, but there have been no palynological studies employing electron microscopy. The pollen of the tribe Galegeae, which includes *Oxytropis*, is generally tricolporate having predominantly perforate or finely reticulate tectum types with little variation or specialisation[13]. Such a "stenopalynous" state may explain why most palynologists have not carried out pollen morphological studies on *Oxytropis* by electron microscopy until now.

In the present study the pollen morphology of the four Japanese *Oxytropis* species was examined by scanning electron microscopy (SEM) and the infrageneric variation of pollen morphology was clarified.

Materials and Methods

Pollen was obtained from herbarium specimens housed in the Botanic Garden, Hokkaido Univeristy (see Specimens Investigated). The materials were acetolysed for 3-4 minutes, washed in distilled water, and dehydrated in an ethanol series. The acetolysed pollen was air dried on specimen stubs with 70 % ethanol, sputter-coated with gold, and examined with an MSM 4C-101 SEM. As the measurements are based on less than 10 pollen grains per species by SEM, the values should be understood as approximate. The descriptive terminology used follows mainly Erdtman[14] and Praglowski and

Punt[15].

Results

Pollen descriptions

Oxytropis campestris subsp. *rishiriensis* (Figs. 1-6)

Pollen grains 3-colporate, prolate. P (polar diameter)=33-34 μm, E (equatorial diameter)=22-24 μm; P/E=1.40-1.46. Amb circular to rounded triangular. Apocolpium diameter 11-13 μm. Colpi 26-29 μm long, 1.2 μm wide, with rather long (5-6 μm) equatorial constrictions (cf. constricticolpate, Erdtman[14]) with more or less obtuse ends. Colpus membrane granular.

Exine perforate to reticulate. In mesocolpia, perforations with a maximum diameter of about 0.8 μm, solitary, elliptical or fused in groups of 2, forming narrow and short irregular shapes, 15-20 per 25 μm^2; reduced in number and size towards the colpi; enlarged in size (a maximum diameter up to 1.2 μm) in the intermediate areas between the mesocolpia and apocolpia. In apocolpia, perforations with a maximum diameter of about 0.4 μm, solitary and circular in shape and sparsely spaced, 15-20 per 25 μm^2.

O. japonica var. *sericea* (Figs. 7-12)

Pollen grains 3-colporate, prolate. P=27-30 μm, E=18-20 μm; P/E=1.38-1.54. Amb circular. Apocolpium diameter 9-10 μm. Colpi 21-23 μm long, 1.2 μm wide, with rather long (4-5 μm) equatorial constrictions, with more or less pointed ends. Colpus membrane with sparsely spaced or indistinct granules.

Exine perforate to reticulate. In mesocolpia, perforations with a maximum diameter of more than 1 μm, solitary, elliptical, or fused in groups of 2-3, forming broad and sometimes long irregular shapes, 25-30 per 25 μm^2; reduced in number and size towards the colpi, especially their equatorial margins; enlarged in size (a maximum diameter up to 1.2 μm) in the intermediate areas between the mesocolpia and apocolpia. In apocolpia, perforations with a maximum diameter of about 0.5 μm, solitary and circular in shape, 25-30 per 25 μm^2.

O. megalantha (Figs. 13-18)

Pollen grains 3-colporate, (subprolate) to prolate. P=32-36 μm, E=23-25 um; P/E=1.32-1.54. Amb circular to rounded triangular. Apocolpium diameter 9-12 μm. Colpi 23-30 μm long, 1.7 μm wide, with rather short (2 μm) equatorial constrictions, with more or less blunt and indistinct ends. Colpus membrane granular.

Exine perforate to reticulate. In mesocolpia, perforations with a maximum diameter of about 0.5 μm, usually solitary, circular and elliptical, 30-35 per 25 μm^2 reduced in size and number towards the colpi, especially their equatorial margins; enlarged in size (a maximum diameter up to 1.0 μm) in intermediate areas between the mesocolpia and apocolpia. In apocolpia, perforations with a maximum diameter of about 0.7 μm, solitary, circular and elliptic, often fused in groups, forming irregular shapes, 10-15 per 25 μm^2.

O. shokanbetsuensis (Figs. 19-24)

Pollen garins 3-colporate, prolate. P=37-40 μm, E=25-28 μm; P/E=1.44-1.57. Amb rounded triangular to 3-lobate. Apocolpium diameter 13-15 μm. Colpi 28-31 μm long, 2 μm wide, without distinct equatorial constrictions (or easily broken by acetolysis treatment). Ora exposed, 6-7 μm long. Ends of colpus more or less obtuse. Colpus membrane with sparcely spaced granules.

Exine perforate. In mesocolpia, perforations with a maximum diameter of usually less than 0.2 μm, solitary, circular or elliptical, and sometimes fused in groups of 2-3, sparsely spaced, about 20 per 25 μm^2. In apocolpia, perforations with a maximum diameter of 0.2 μm, circular and elliptic,

more sparsely spaced, about 15 per 25 μm^2.

Pollen types

Two pollen types are distinguished based on exine structure, aperture details and grain size.

Key to pollen types:

A. Pollen grains with perforate to reticulate exine structure, perforations with a maximum diameter of more than 0.8 μm, colpi with distinct equatorial constrictions (bridges) covering ora, polar diameter 27-36 μm..... *O. campestris*-type
(*O. campestris* subsp. *rishiriensis, O. japonica* var. *sericea, O. megalantha*)

AA. Pollen grains with perforate exine structure, perforations with a maximum diameter of 0.3 μm, colpi without distinct equatorial constrictions, ora exposed, polar diameter 37-40 μm ..... *O. shokanbetsuensis*-type (*O. shokanbetsuensis*)

Discussion

According to Ikuse, the tricolporate pollen grains (26-27.5 × 22-23.5 μm) of *Oxytropis japonica* var. *japonica* have a reticulate pattern with the lumina of less than 0.8 μm in diameter [16] or subreticulate with the lumina of 1 μm in diameter [17]. Bouda [18] reported that *Oxytropis japonica* var. *japonica* has spherical tricolporate grains (35 × 35 μm) with a reticulate pattern and the lumina of 0.6 μm in diameter and *O. japonica* var. *sericea* and O. *megalantha* have prolate tricolporate grains (24 × 12 μm and 25 × 15 μm, respectively) with a more fine reticulate pattern. Ikuse and Bouda did not acetolyze the pollen grains, so we can not directly compare their values with ours, especially considering the P/E ratios described by Bouda[18], which may be based on the shrunken grains. As the pollen of *Oxytropis japonica* var. *japonica* was not examined in the present study, the pollen morphological differences between the two varieties of *Oxytropis japonica* indicated by Bouda[18] was not ascertained. In the present study, the pollen of *Oxytropis megalantha* had more fine perforations in mesocolpia than that of O. *japonica* var. *sericea*.

Oxytropis shokanbetsuensis was distinguished from other Japanese *Oxytropis* species by having pollen grains with more fine perforation exine structures and indistinct equatorial constrictions of the colpi. Furthermore, the pollen grains of *Oxytropis shokanbetsuensis* were the largest in size among the four *Oxytropis* species examined here.

This species was described by Miyabe and Tatewaki [19] in 1935 as an endemic species to Mt. Shokanbetsu, Hokkaido, northern Japan. They regarded it as being related to *O. hidaka-montana* Miyabe et Tatewaki. In 1975, Toyokuni [20] regarded it as a subspecies of *Oxytropis retusa* of the Kurile Islands; i. e., *Oxytropis retusa* Matsumura subsp. *shokanbetsuensis* (Miyabe et Tatewaki) Toyokuni, but except him, all Japanese taxonomists recognized it to be an endemic species to Mt. Shokanbetsu. As revealed in this study, distinct pollen morphology of *Oxytropis shokanbetsuensis* among Japanese *Oxytropis* species may support its endemic status. Further critical macromorphological studies should be conducted for this species.

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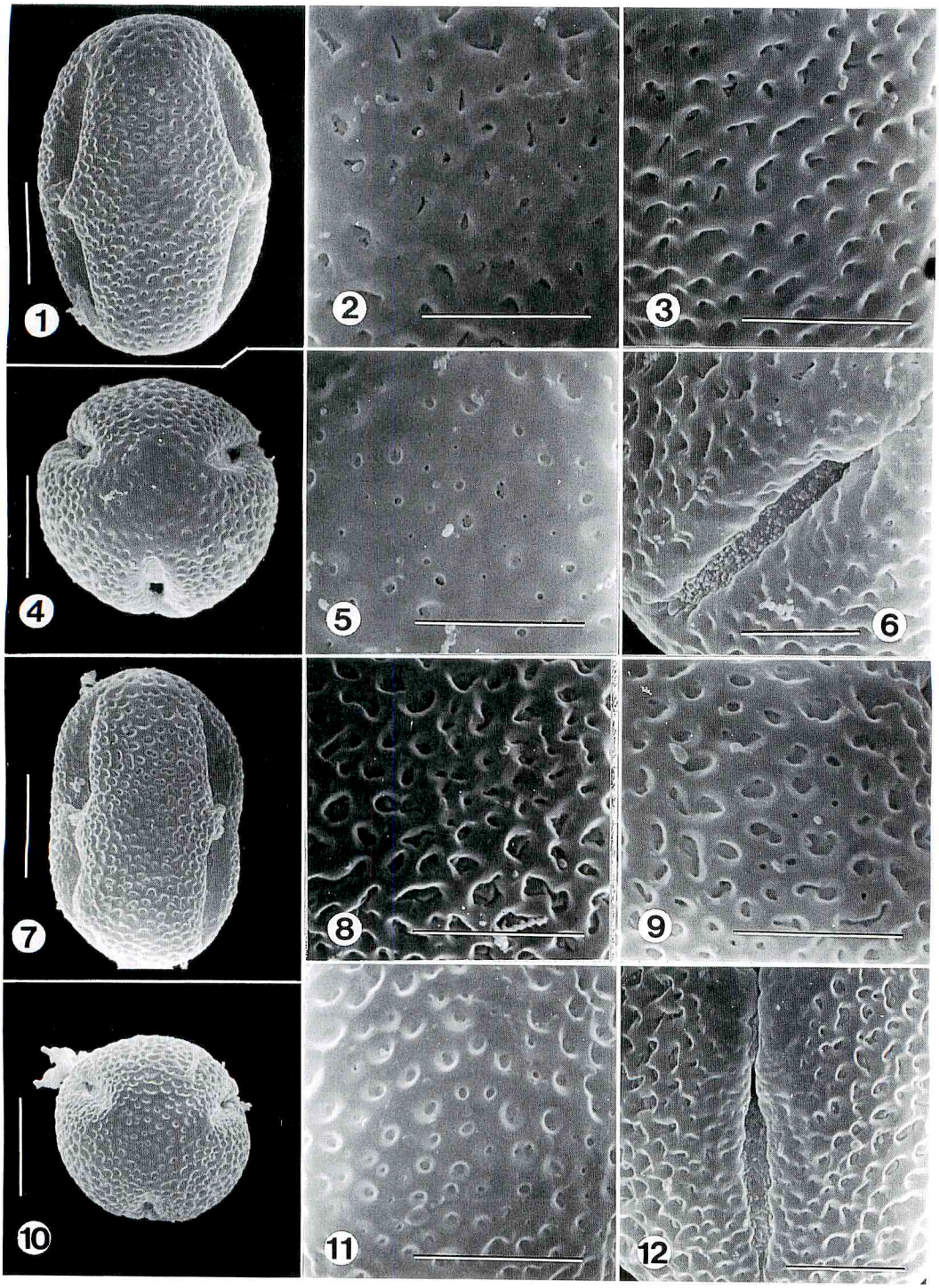
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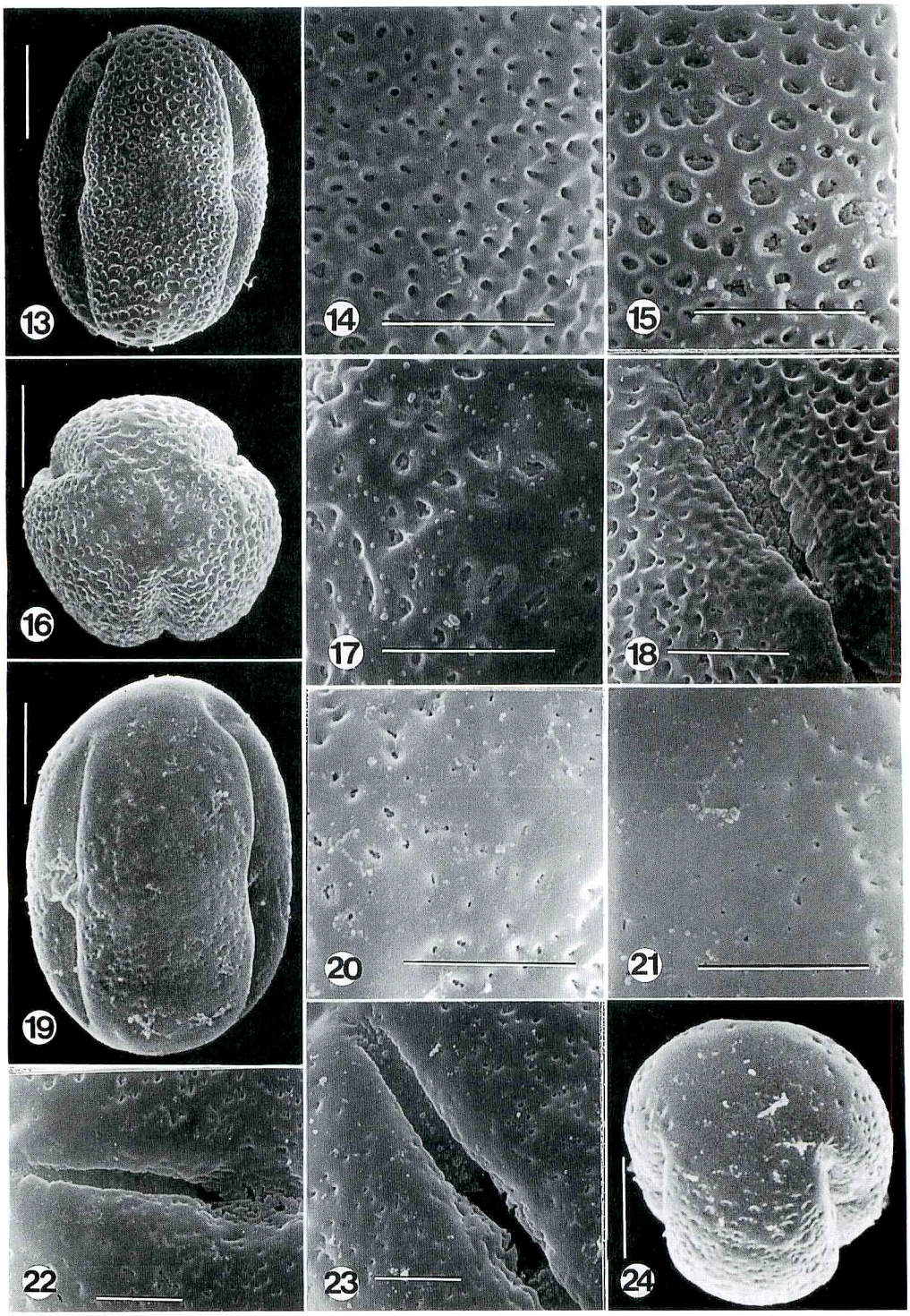
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Legends for Figures

Figs. 1-6. *Oxytropis campestris*. 1. Equatorial view showing mesocolpium. 2. Detail of exine at mesocolpium. 3. Oblique view showing detail of exine at mesocolpium to apocolpium. 4. Polar view. 5. Detail of exine at apocolpium. 6. Detail of colpus.
Figs. 7-12. *Oxytropis japonica*. 7. Equatorial view showing mesocolpium. 8. Detail of exine at mesocolpium. 9. Detail of exine at mesocolpiun to apocolpium. 10. Polar view. 11. Detail of exine at apocolpium. 12. Detail of colpus. Scale bars=10 μm in Figs. 1, 4, 7 and 10; 5 μm in the other Figs.

Figs. 13-18. *Oxytropis megalantha*. 13. Equatorial view showing mesocolpium. 14. Detail of exine at mesocolpium. 15. Detail of exine at mesocolpium to apocolpium. 16. Polar view. 17. Detail of exine at apocolpium. 18. Detail of colpus.
Figs. 19-24. *Oxytropis shokanbetsuensis*. 19. Equatorial view showing mesocolpium. 20. Detail of exine at mesocolpium. 21. Detail of exine at mesocolpium on another grain. 22. Detail of colpus. 23. Detail of colpus on another grain. 24. Oblique polar view. Scale bars=10 μm in Figs. 13, 16, 19 and 24; 5 μm in the other Figs.

Specimens Investigated

Scientific names follow Ohashi[2]. Voucher specimens are preserved in the Herbarium of the Botanic Garden, Faculty of Agriculture, Hokkaido University.
Oxytropis campestris (L.) DC. subsp. *rishiriensis* (Matsum.) Toyokuni JAPAN. Hokkaido, Mt. Yubari, July 19, 1983, No. 4397.
O. japonica Maxim. var. *sericea* Koidz. JAPAN. Hokkaido, Mt. Taira-yama, June 30, 1982, No. 2640.
O. megalantha H. Boiss. JAPAN. Hokkaido, Isl. Rebun, June 29, 1983, No. 4328.
O. shokanbetsuensis Miyabe et Tatew. JAPAN. Mt. Shokanbetsu, July 28, 1983, No. 4506.

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日本産ゲンゲ属（マメ科）の花粉形態

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日本産ゲンゲ属植物4種の花粉形態を走査型電子顕微鏡で観察した．本属の花粉は長球形で内口式三溝型の開口部をもち，表面模様は穿孔から網目型である．4種の中では，暑寒別岳の固有種マシケゲンゲが特徴的な花粉形態を持ち区別できる．その特徴は花粉のサイズが大きく，溝の中くびれ部が不明瞭，花粉壁表面は細かい穿孔（直径0.3μm以下）を持つの諸点である．