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Scanning Electron Microscope Observation of Leaf Surface of *Hydrangea macrophylla*

By

Yoshihiko SATÔ* and Masayuki TANAKA*

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Abstract. There are many intraspecific botanical entities in *Hydrangea macrophylla*. In the followings of them, the surface structure of their leaves was investigated.

(1) *Hydrangea macrophylla* var. *macrophylla* forma *macrophylla*
(2) *H. macrophylla* var. *macrophylla* forma *normalis*
(3) *H. macrophylla* var. *megacarpa* forma *megacarpa*
(4) *H. macrophylla* var. *acuminata* forma *acuminata*
(5) *H. macrophylla* var. *angusta* forma *angusta*

In the leaves of *H. macrophylla* group, variations which can be expected to have a taxonomic and phylogenetic significance were found. Forma *macrophylla* and forma *normalis* had few trichomes, and each of their adaxial epidermal cells had not any grooves and ridges on its exposed surface. On the other hand, forma *megacarpa*, forma *acuminata* and forma *angusta* much commonly had trichomes, and each of their adaxial epidermal cell had many fine grooves and ridges on its exposed surface. Only forma *megacarpa* of the members examined had trichomes with a hillock-like structure at their base, while four other members examined had trichomes without a hillock-like structure at their base. Taxonomic treatment of *H. macrophylla* will have to be reviewed in future, after many species of which the genus *Hydrangea* is composed have been examined in detail and compared with each other from botanic points of view.

Introduction

The genus *Hydrangea* is treated either as one of genera of which the Saxifragaceae (s. l.) are composed (Hooker, 1865; Engler, 1928; Thorne, 1976; etc.) or as one genus of the family Hydrangeaceae which is one of families established by subdividing the Saxifragaceae (s. l.) into small ones (Hutchinson, 1956; Airy Shaw, 1966; Takhtajan, 1980; Cronquist, 1981; etc.).

Anatomical characters of vegetative organs in *Hydrangea* have been occasionally described by Metcalfe & Chalk (1950), Stern (1978), Satô (1989) and so on. In particular, Stern (1978) reported on foliar and nodal anatomy and the structure of the secondary xylem in 14 species of *Hydrangea*. He pointed out in his paper that there are some distinct differences in anatomical character between section *Hydrangea* and section *Cornidia*. Satô (1989), senior author of

* Department of Biology, Faculty of Education, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama, 240, Japan.

the present paper, has described anatomical characters found in the transverse section of foliage leaves of *Hydrangea macrophylla* group, and presented that there are distinct differences in foliar anatomy even among the intraspecific botanical entities. Furthermore, he has inferred that some intraspecific variations may occur in the surface view of leaves of *H. macrophylla* group. We have intended to observe the leaf surface of *H. macrophylla* group using a scanning electron microscope (SEM).

Material and Method

There are many intraspecific botanical entities in *Hydrangea macrophylla*. In the followings of them, the surface structure of their leaves was investigated.

(1) *Hydrangea macrophylla* var. *macrophylla* forma *macrophylla*
(2) *H. macrophylla* var. *macrophylla* forma *normalis*
(3) *H. macrophylla* var. *megacarpa* forma *megacarpa*
(4) *H. macrophylla* var. *acuminata* forma *acuminata*
(5) *H. macrophylla* var. *angusta* forma *angusta*

Hydrangea macrophylla is a deciduous, soft-wooded shrub. Cauline foliage leaves which had fully grown were collected from September to November in 1988. But, in forma *macrophylla* and forma *normalis*, very young foliage leaves as well as their fully-grown leaves were collected, because trichomes were very meager on the surface of their fully-grown leaves. The leaves of forma *macrophylla*, forma *normalis* and forma *angusta* were collected from individuals planted ornamentally in Fujisawa City, Kanagawa Prefecture, from ones growing wild at Enoshima Islet, Fujisawa City, and from ones growing wild near Hacchou Pond in Mt. Amagi, respectively. Twigs of forma *megacarpa* and forma *acuminata* for making cuttings were collected at Mt. Hakkoda Botanical Laboratory of Tôhoku University, Aomori Prefecture in 1983 and at Kiyosato, Yamanashi Prefecture in 1987, respectively. They were planted and grown on the campus of the Yokohama National University. The leaves of them were collected from the individuals grown from the cuttings.

These leaves collected were sectioned into about five millimeters square with razor blades. The small pieces of leaves were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, each in 0.1 M phosphate buffer (pH 7.2-7.4). After the fixed material was dehydrated in a graded ethanol series, ethanol was replaced with amil acetate. The material was critical-point dried using carbon dioxide as the transition fluid. The leaf pieces were mounted on a stub using a double-stick tape, and sputter-coated with platinum. The coated material was viewed and photographed with a JEOL JSM T-100 scanning electron microscope. The length and the height of structures and the area of cell surface were measured from photographs using the image analysis program (Nikon Cosmozone 1S).

Observation

(1) Stomata

Stomata (Figs. 1A, 2A, 3A, 4A) were present only on the abaxial side of leaves of five members examined but they were absent on the adaxial side. In forma *megacarpa*, there were a few immature stomata (Fig. 2B) among the mature ones on the abaxial surface of the leaves collected at the beginning of September. There was no difference in structure of the stomata among any five members. There was, however, a difference in the long length of their outer stomatal ledge aperature as shown in Table 1. The average length of

Table 1. Long length (μm) of outer stomatal ledge aperture.

	Average length	Minimum length	Maximum length
forma *macrophylla*	10.7	6.2	16.8
forma *normalis*	9.9	5.7	15.2
forma *megacarpa*	11.9	6.9	16.6
forma *acuminata*	16.2	10.4	22.9
forma *angusta*	13.9	8.1	19.8

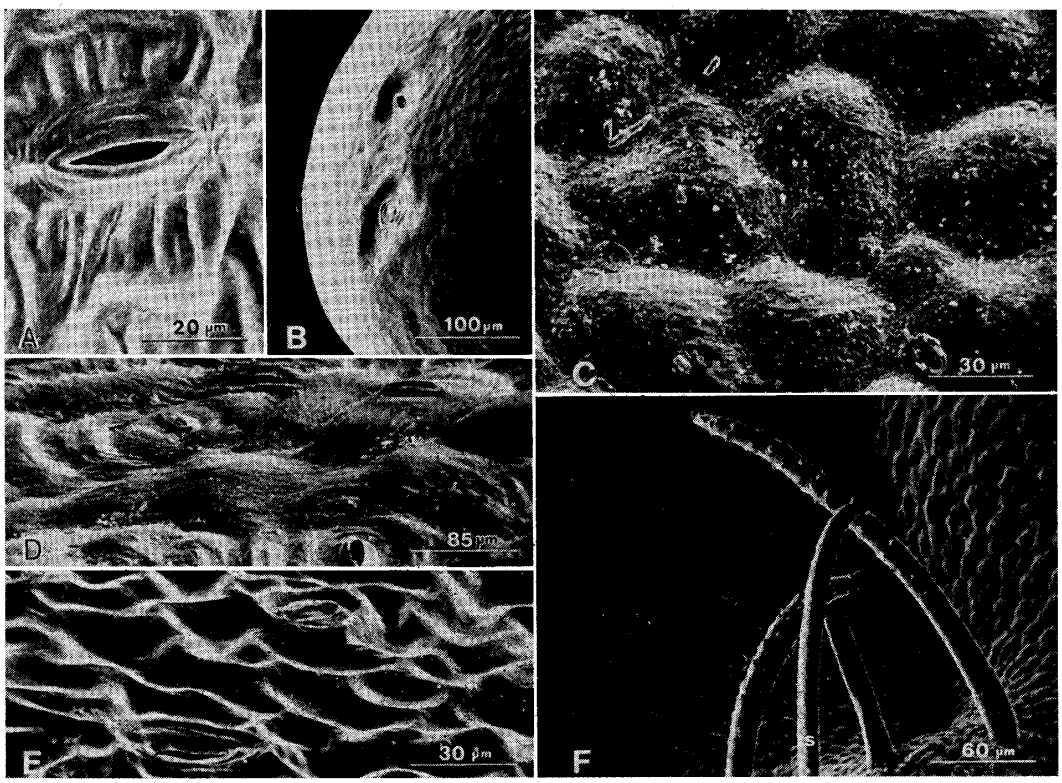

Fig. 1. *Hydrangea macrophylla* var. *macrophylla* forma *macrophylla* (A, E, F) and forma *normalis* (B, C, D). A. Stoma. B. Hydathodes on tooth surface of leaf margin. C. Adaxial epidermis. D. Nearly smooth abaxial epidermis. E. Relatively irregular abaxial epidermis. F. Trichomes of very young leaf. Note a trichome (s) with the nearly smooth surface.

forma *macrophylla* is similar to that in forma *normalis*. Their average length is relatively short and it is about two-thirds the length of forma *acuminata* which has the longest aperture among all members measured. The average length of forma *megacarpa* and forma *angusta* is the intermediate value of length among the all members measured.

(2) Hydathodes

The leaves of all members examined had a toothed margin. In all members, a few hydathodes (Fig. 1B) were present on a surface of some teeth along the leaf margin, not that they were present on the surface of all teeth along the leaf margin. The hydathodes looked like a crater enclosed with a slightly elevated rim, but the crater might be open or not.

(3) Epidermal cells

The venation of leaves of all members examined was pinnate, and the secondary veins and their branched veins formed meshes delimiting small areoles. In face view on both adaxial and abaxial sides, the epidermal cells associated with the veins were narrow and elongated along the veins.

In forma *macrophylla* and forma *normalis*, the epidermal cells associated with the areoles (Fig. 1C) had a relatively simpler outline in face view than the other three members had. The values of area enclosed with the outline of each abaxial epidermal cell associated with areoles are shown in Table 2; the average values in forma *macrophylla*, forma *normalis* and forma *megacarpa* are relatively similar to each other, while those in forma *acuminata* and forma *angusta* are very similar to one another, too. The average values of the latter are about two times the values of the former. The exposed whole surface of both adaxial and abaxial epidermis (Figs. 1C, D) usually was rather smooth in forma *macrophylla* and forma *normalis*, though in the former, some of the leaves examined have relatively irregular abaxial epidermis (Fig. 1E). In the two, subsidiary cells usually had several folds at their exposed surface (Fig. 1A).

In face view of both adaxial and abaxial epidermis of leaves of forma *acuminata* (Fig. 3D) and forma *angusta* (Fig. 4D), the outline of each epidermal cell associated with areoles was complicate and deeply or slightly lobed. Each of their adaxial epidermal cells (Figs. 3B, 4B) had many fine distinct grooves and ridges on its exposed surface. In forma *acuminata*, each of ridges was continuous (Fig. 3B), while in forma *angusta*, it broke into small or short ridges

Table 2. Area (μm^2) enclosed with outline of each abaxial epidermal cell associated with areoles.

	Average area	Minimum area	Maximum area
forma *macrophylla*	884.0	632.3	1192.1
forma *normalis*	710.7	523.6	889.5
forma *megacarpa*	736.6	407.8	957.7
forma *acuminata*	1527.7	792.0	2601.7
forma *angusta*	1498.3	733.4	2219.4

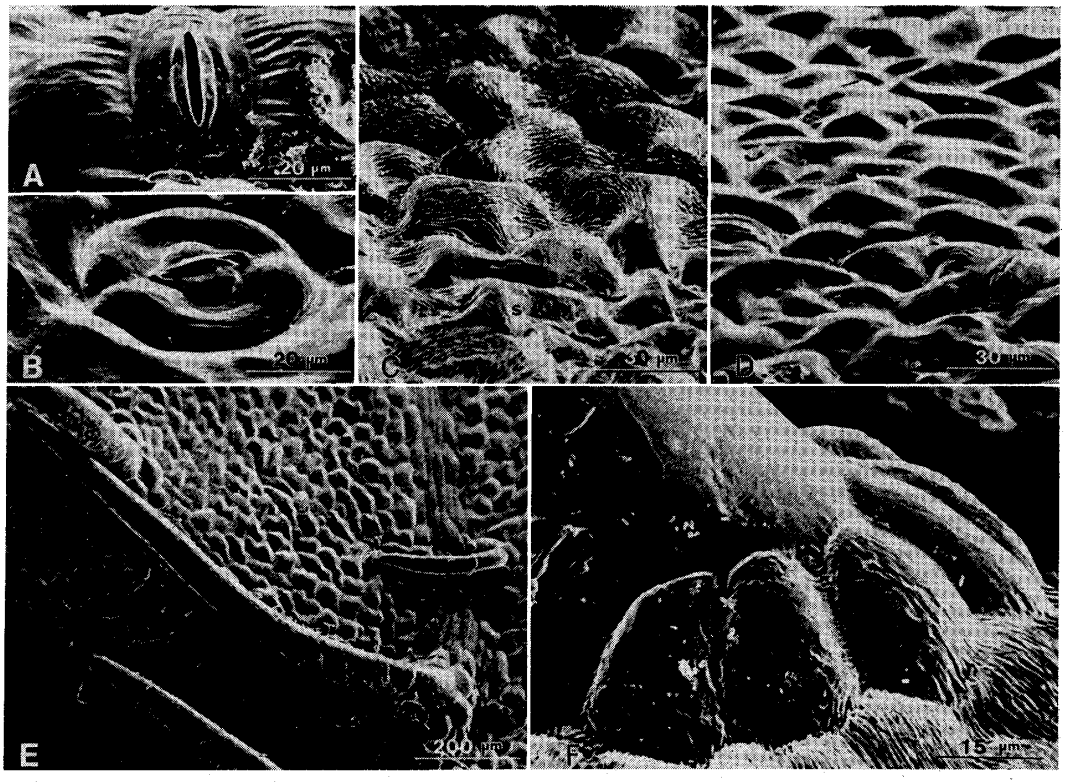

Fig. 2. *Hydrangea macrophylla* var. *megacarpa* forma *megacarpa*. A. Stoma. B. Hydathode. C. Adaxial epidermis. Note epidermal cells (s) without grooves and ridges at their exposed surface. D. Relatively irregular abaxial epidermis. E. Trichomes on adaxial surface. F. Hillock-like structure at trichome base.

(Fig. 4B). Therefore, the latter had more complicate surface appearance of the adaxial epidermis than the former had. However, it remains to be solved whether these grooves and ridges are formed by the cell wall itself or they are formed by the epicuticular wax. On the other hand, every abaxial epidermal cell of forma *acuminata* (Fig. 3C) and forma *angusta* (Fig. 4C) usually had not grooves and ridges on its exposed surface. That is, the surface of abaxial epidermal cells were smooth. However, some of the subsidiary cell and the cells adjacent to them had several folds at their exposed surface (Figs. 3A, 4A). Their folds were almost similar to those at the surface of these cells in forma *macrophylla* and forma *normalis*. These folds seem to be artificially formed during fixation, dehydration or/and drying. In forma *acuminata* and forma *angusta*, almost all epidermal cells on both the adaxial and abaxial sides were invested with a thin cell wall, and they greatly swelled outwardly (Figs. 3B, C, 4B, C). Therefore, the whole surface of the epidermis on both sides was very irregular.

In face view of forma *megacarpa*, also, the outline of the epidermal cells on both sides was deeply or slightly lobed. The whole surface of both the adaxial and abaxial epidermis was rather irregular. The irregularity of abaxial epidermis was similar to that found occasionally in forma *macrophylla*. In

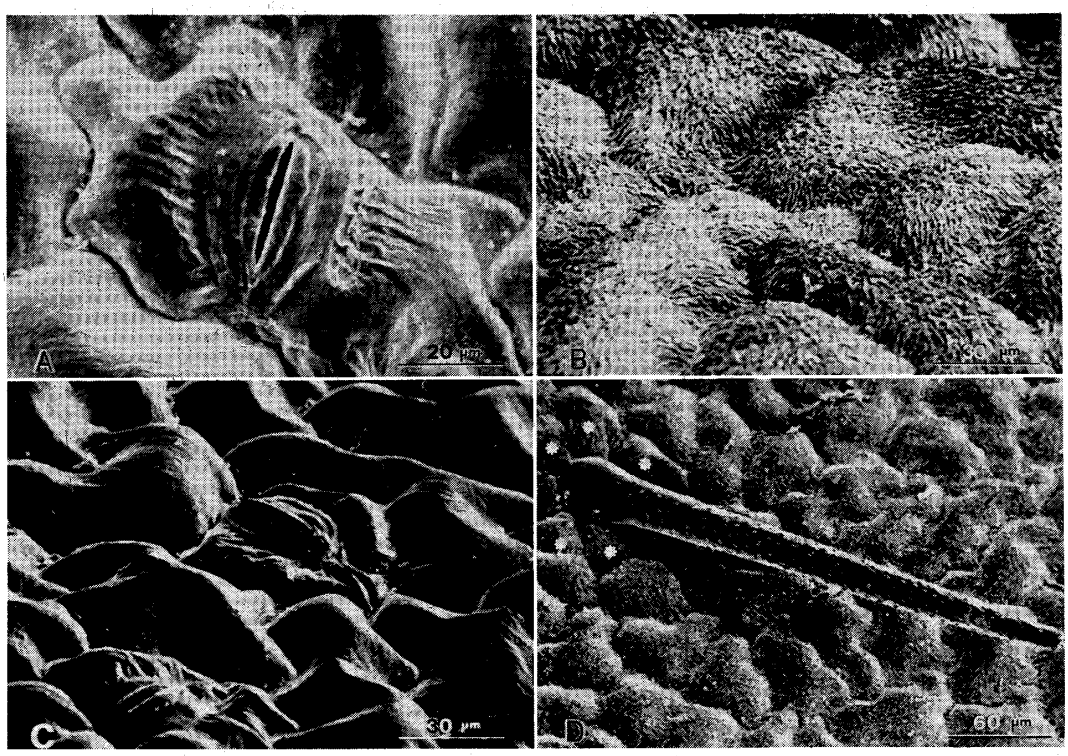

Fig. 3. *Hydrangea macrophylla* var. *acuminata* forma *acuminata*. A. Stoma. B. Irregular adaxial epidermis. C. Irregular abaxial epidermis. D. Trichome with radially arranged cells (*) at its base.

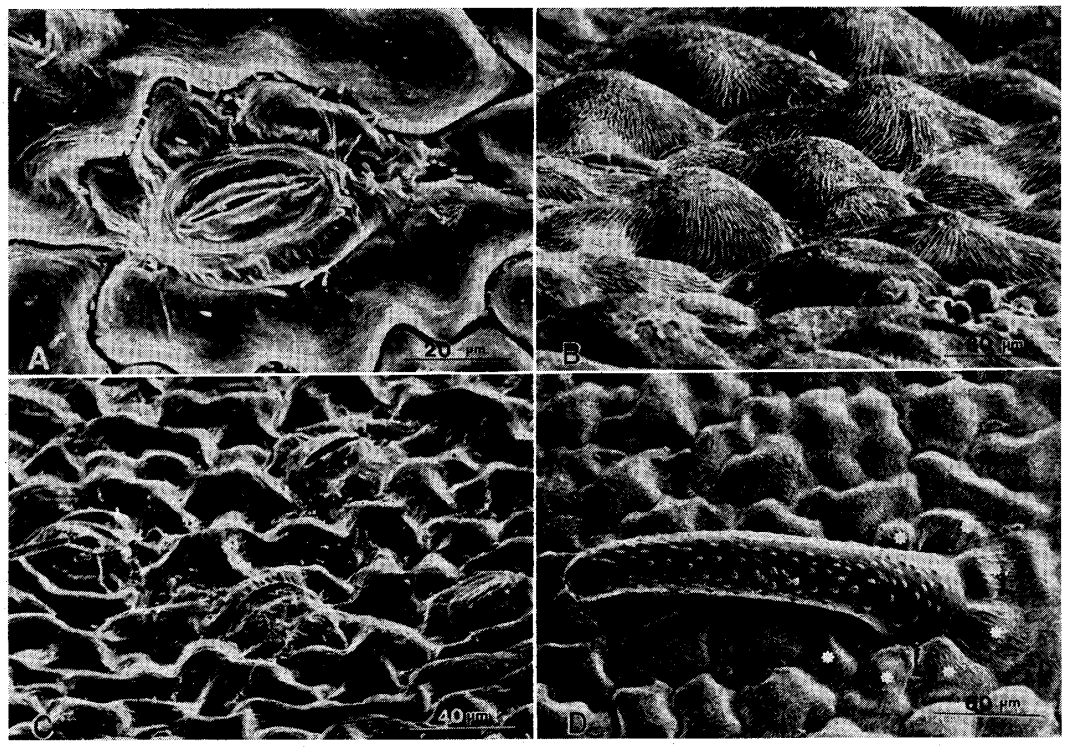

Fig. 4. *Hydrangea macrophylla* var. *angusta* forma *angusta*. A. Stoma. B. Irregular adaxial epidermis. C. Irregular abaxial epidermis. D. Trichome with radially arranged cells (*) at its base.

forma *megacarpa*, also, usually its adaxial epidermal cells had many fine grooves and ridges at their exposed surface (Fig. 2B). The ridge was continuous without any breaking into small or short ridges and without crossing of adjacent ridges. That is, the surface condition of forma *megacarpa* was more similar to that of forma *angusta* than that of forma *acuminata*. In forma *megacarpa*, however, some of the epidermal cells had the exposed surface at which grooves and ridges were partially absent (Fig. 2B). Thus, it seems that the exposed surface of leaf epidermis in forma *megacarpa* has features common to that of forma *macrophylla* as well as to that of forma *angusta*.

(4) Trichomes

Trichomes, usually, occurred on both surfaces of the leaf and they were more prevalent on the abaxial surface than on the adaxial surface. In forma *macrophylla* and forma *normalis*, however, they were very meager, and they were usually distributed only on the surface of vein. In our examination for fully-grown leaves in the two, the trichomes were not viewed on other areas of the leaf surface. Furthermore, some of the leaves examined had not any trichomes through the whole area of the leaf surface. A trichome of their mature leaf usually had many small protuberances at its surface and it was usually 150–250 μm long. But, in their young developing leaf (Fig. 1F), rarely, there were nearly smooth-walled trichomes among the tubercular ones.

In forma *megacarpa*, forma *acuminata* and forma *angusta*, a trichome was relatively common at the surface of the interveinal areas as well as at the surface of the veins. In particular, forma *megacarpa* most commonly had trichomes among all members examined. In forma *megacarpa* (Fig. 2E) and forma *acuminata* (Fig. 3D), their trichomes were usually more than 300 μm long and in the former, some of them attained to 800 μm long. On the other hand, forma *angusta* (Fig. 4D) usually had short trichomes on the surface of veins and interveins. The trichomes usually were less than 200 μm long and the trichomes with more than 200 μm in length were meager.

The trichomes of forma *megacarpa* (Fig. 2E), forma *acuminata* (Fig. 3D) and forma *angusta* (Fig. 4D) invariably had many small protuberances at their surface. The protuberances of forma *megacarpa* and forma *acuminata* usually were higher than those of forma *angusta*; usually, the former usually had the protuberances of about 4 μm high and the latter had those of less than 2 μm high.

There was a difference in the appearance of epidermal cells situated at the base of trichomes. In forma *megacarpa* (Figs. 2E, F), the base of trichome was surrounded with several epidermal cells which were turgid to raise above the level of the epidermis. And these turgid cells arranged radially around the trichome base. That is, a hillock-like structure was formed at the trichome base. In forma *megacarpa*, trichomes on the vein also had a ring of turgid-appearing epidermal cells at their base. In forma *acuminata* (Fig. 3D) and forma *angusta* (Fig. 4D), the epidermal cells at the base of their trichomes in the

interveinal areas arranged radially to form a ring, but they were not turgid. That is, they had no hillock-like structure at their base. In this respect, the trichomes of forma *acuminata* and forma *angusta* were different from the trichome of forma *megacarpa*. Special cellular arrangement and structure were absent at the base of trichomes on the leaf vein in the members except for forma *megacarpa*.

Discussion

In all members examined here, stomata are present only at the abaxial epidermis and there is no difference in their structure and type. Hydathodes are present on the surface of teeth along the leaf margin. Thus, there are not a few features common to all members examined. However, the diversity which can be expected to have a taxonomic and phylogenetic significance is found among the surface structure of leaf of all members examined.

Five kinds of plants examined are divided into two groups as follows:

Group A: *H. macrophylla* var. *macrophylla* forma *macrophylla*
H. macrophylla var. *macrophylla* forma *normalis*

Group B: *H. macrophylla* var. *megacarpa* forma *megacarpa*
H. macrophylla var. *acuminata* forma *acuminata*
H. macrophylla var. *angusta* forma *angusta*.

In two members of Group A, trichomes are very meager on the surface of their fully-grown leaf. They usually have many small protubelances on their surfaces, though the trichomes with a nearly smooth surface are rarely present, too. The whole surface of the adaxial and abaxial epidermis of the two members usually is nearly smooth, though there occasionally are leaves with rather irregular abaxial epidermis in forma *macrophylla*. Every cell of their adaxial epidermis has not any grooves and ridges at its exposed surface. On the other hand, in three members of Group B, trichomes are relatively common. They invariably have many small protubelances at their surfaces. Every cell of the adaxial epidermis in the three has many fine grooves and ridges on the exposed surface, though the discontinuous ridges of forma *acuminata* differ from the continuous ones of forma *megacarpa* and forma *angusta*. But, some epidermal cells of forma *megacarpa* partially have not grooves and ridges at their surface. The whole surface of both adaxial and abaxial epidermis of forma *acuminata* and forma *angusta* is distinctly irregular, and in forma *megacarpa* also the epidermis has an rather irregular surface. That is, three members of Group B have an irregular abaxial epidermis. In these respects, the three members of Group B have some common features of leaf surface which differ from the features of the two members of Group A. However, it should received much attention that forma *megacarpa* assigned to Group B has the features common to those of Group A.

A trichome with a hillock-like structure is prevalent through section *Hy-*

drangea of the genus *Hydrangea* (Stern, 1978). But, this type of the trichomes is present only in forma *megacarpa*, and it is absent even in forma *acuminata* and forma *angusta* which have salient features common to forma *megacarpa*. In this respect, Group B is subdivided into two subgroups as follows:

Subgroup B1: *H. macrophylla* var. *megacarpa* forma *megacarpa*
Subgroup B2: *H. macrophylla* var. *acuminata* forma *acuminata*
H. macrophylla var. *angusta* forma *angusta*.

The rare occurrence of trichome with smooth surface in Group A seems to link a trichome feature in section *Hydrangea* with that in section *Cornidia* in which a trichome has a smooth surface (Stern, 1978). In forma *megacarpa*, some epidermal cells partially have not the grooves and ridges at their exposed surface of the adaxial epidermis, and in spite of the small values of areas enclosed with the outline of the abaxial epidermal cells associated with the areoles in face view, the outer stomatal ledge aperture has an intermediate value in its long length. These features found in forma *megacarpa* seem to link a feature of leaf surface in Group B with that in Group A.

Both of forma *macrophylla* and forma *normalis* are precisely different in foliar anatomy from three other members examined (Satô, 1989). This examination of ours shows that there are some intermediate features among features found in leaves of all members examined here. Occurrence of some intermediate features seems to show that the features found in the foliar structures have a taxonomic or phylogenetic significance. The scientific names used in our paper are ones which have been chosen by Ohwi (1965). We consider that these names must be reviewed in future, based on the detail examination and comparison of many other features among the intraspecific entities assigned to *H. macrophylla* and the detail examination and comparison of various features among species of which the genus *Hydrangea* is composed.

摘　　要

広義のアジサイ（*Hydrangea macrophylla*）には多くの種内分類群が記載されている。その内，次の5種類の成熟した葉の表面構造について比較することを試みた。

(1) アジサイ　*H. macrophylla* var. *macrophylla* forma *macrophylla*
(2) ガクアジサイ　*H. macrophylla* var. *macrophylla* forma *normalis*
(3) エゾアジサイ　*H. macrophylla* var. *megacarpa* forma *megacarpa*
(4) ヤマアジサイ　*H. macrophylla* var. *acuminata* forma *acuminata*
(5) アマギアマチャ　*H. macrophylla* var. *angusta* forma *angusta*

アジサイ（狭義）はガクアジサイを鑑賞用に改良して作り出されたものと言われているが，両者の葉の表面の構造には特筆すべき特徴の違いは見いだせなかった。しかし，他の3種類の葉の表面構造は，明らかにアジサイ（狭義）やガクアジサイとは異なった特徴を示していた。アジサイ（狭義）とガクアジサイの葉には毛状体はほとんど見られず，向軸側の表皮細胞の表面はほぼ平滑で特別な構造は見いだせなかった。しかし，エゾアジサイやヤマアジサイ，アマギアマチャの葉にはかなりよく毛状体が見られ，向軸側の表皮細胞の表面には特徴的な非常に細かい畝と溝からなる縞模様が

見られた。エゾアジサイの毛状体は，今回調査した他の4種類では見られない小丘状の構造をその基部に持っていた。この小丘状の構造を持つ毛状体は，アジサイ（広義）の収容されているアジサイ節には広く存在しているものである (STERN, 1978)。つまり，小丘状の構造のない毛状体がアジサイ（広義）では普遍的であり，小丘状構造を持つ毛状体は少ないという点では，アジサイ(広義)はアジサイ節のなかで特殊な存在であり，アジサイ（広義）の中では小丘状の構造を持つ毛状体を持つエゾアジサイは特筆されるべき存在である。この論文では大井（1965）によって採択されている学名を用いたが，*Hydrangea macrophylla* という学名の下にまとめられている分類群の分類学的な扱いは，アジサイ属の種間やアジサイ（広義）の種内での植物学上の多くの特徴を調査したのちに，再検討されるべきであろう。

この研究には，昭和63年度の教育研究特別経費（「電子顕微鏡を利用した教材開発のための総合的研究」—研究代表者：松田忠男教授—）から一部援助していただいたことをここに付記し，感謝の意を表したい。

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