National Research
Council Canada

Conseil national
de recherches Canada

NRC · CNRC

Reprinted from

Canadian
Journal of
Botany

Réimpression de la

Revue
canadienne
de botanique

Anisophylly in *Aucuba japonica* (Cornaceae): An outcome of spatial crowding in the bud

Md. Sohrab Ali and Kihachiro Kikuzawa

Volume 83 • Number 2 • 2005

Pages 143–154

Canada

Anisophylly in *Aucuba japonica* (Cornaceae): An outcome of spatial crowding in the bud

Md. Sohrab Ali and Kihachiro Kikuzawa

Abstract: Anisophylly in *Aucuba japonica* Thunb. occurs exclusively in axillary buds on shoots of individuals that have reached reproductive maturity; juvenile plants or sprouts and shoots from terminal vegetative buds on adult plants are isophyllous. The initially smaller leaf primordium in each anisophyllous leaf pair gives rise to the larger final leaf size. Immediately before bud break, the size of the initially smaller primordium is already almost twice as large as the initially larger primordium, and the size differential is further amplified following bud break. The degree of anisophylly, however, varies among the nodes of a shoot, depending on leaf pair orientation (tangential or parallel to the inflorescence axis) and nodal position. Paired leaves approach isophylly when they originate tangential to an inflorescence but become anisophyllous when originating parallel to an inflorescence. The degree of anisophylly depends on crown light conditions through effects on terminal bud size and foliage distribution on the shoot, but ultimately appears to arise from space limitations and crowding between the leaf primordia and the developing inflorescence in terminal reproductive buds.

Key words: Aucuba, anisophylly, bud internal morphology, developmental anatomy, developmental constraints.

Résumé : Chez l'*Aucuba japonica* Thunb., l'anisophyllie survient uniquement dans les bourgeons axillaires sur des tiges d'individus qui ont atteint la maturité reproductive; les plantes juvéniles et les rejets ainsi que les tiges issues du bourgeon terminal sur plantes adultes sont isophylles. Le primordium foliaire plus petit au départ de chaque paire de feuilles anisophylles atteint la dimension foliaire finale la plus grande. Immédiatement avant l'ouverture du bourgeon, la dimension du plus petit primordium foliaire au départ, est déjà presque le double de celle du primordium plus grand, et cette différence de dimension s'amplifie suite à l'ouverture du bourgeon. Cependant, le degré d'anisophyllie varie entre les noeuds d'une tige, selon l'orientation de la paire de feuilles (tangentielle ou parallèle à l'axe floral) et la position nodale. La paire de feuille approche de l'isophyllie lorsqu'elles se forment tangentiellement par rapport à l'inflorescence mais devient anisophylle lorsque son origine est parallèle à l'inflorescence. Le degré d'anisophyllie dépend des conditions d'illumination de la cime via ses effets sur la dimension des bourgeons et la distribution du feuillage sur la tige, mais ultimement semble être provoqué par des limitations d'espace et le tassement des primordiums foliaires et des inflorescences en développement, dans les primordiums terminaux reproducteurs.

Mots clés : Aucuba, anisophyllie, morphologie interne des bourgeons, développement anatomique, contraintes au développement.

[Traduit par la Rédaçtion]

Introduction

Anisophylly refers to differences in the size and shape of the two leaves opposite each other at a nodal position (Mueller and Dengler 1984). The degree of anisophylly (White 1955) can be expressed by the magnitude of difference in leaf size. Anisophylly is associated with a range of phyllotactic patterns (Dengler 1999), and is expressed to varying degrees in all or part of the shoot system. Nodes of a shoot can range from strongly anisophyllous to completely isophyllous, depending on position on the shoot and leaf pair orientation (Sánchez-Burgos and Dengler 1988). Anisophyllous leaves are reported to differ histologically (Dengler 1980) and also functionally (Goebel 1900). Development of anisophylly is likely to be regulated by more than one physical or physiological factor, even within a single species (Dengler 1999).

In previous studies, anisophylly has been causally related to distribution of hormones (Schander 1958, as mentioned by Larson and Richards 1981), correlative phenomenon (Dostal 1967), vascular connections (Larson and Richards 1981), shoot apical cell structure (Goebel 1900), light (Figdor 1911, as stated by Fuller 1913), and gravity (Sinnott and Durham 1923). In some species, anisophylly occurs with the switch from juvenile to adult growth (Troll 1937, as quoted by Dengler 1999). The shape and size of the anisophyllous leaves also depend on the diameter of the shoot (Wardlaw 1952; Allsop 1967), which reflects the role of nutrition on growth and development of foliage. The developmental expression of anisophylly appears to be regulated both within the bud and during leaf expansion (Sánchez-

Received 25 May 2004. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 2 February 2005.

M.S. Ali[1] and K. Kikuzawa. Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

[1] Corresponding author (e-mail: sohrab@kais.kyoto-u.ac.jp).

doi: 10.1139/B04-157

Burgos and Dengler 1988). The degree of anisophylly is influenced by irradiance (Mueller and Dengler 1984), varying within and between different light regimes. Variations in the degree of anisophylly by different light regimes possibly occur through affecting shoot nutrition rather than varying leaf thickness (Dengler and Sánchez-Burgos 1988) as light level on anisophyllous leaf surfaces might not differ greatly. Shade-induced clustering of foliage at the tip of the shoot (Givnish 1984) that increased crowding of developing organs in buds influenced the degree of anisophylly, perhaps because of space limitations (Wiehler 1978). Although spatial condition has been suggested to play a role in anisophyllous development (White 1955, 1957; Wiehler 1978), the possibility of spatial constraints as a causal factor for anisophylly remains unexplored and has not been tested before through direct manipulation of space in a developing bud. For space constraints to lead to anisophylly, space should be differently available for growth of the two primordia (at least for decussate phyllotaxis). Primordial size and mode of development have received attention (Sánchez-Burgos and Dengler 1988; Goebel 1900), but not the spatial arrangement of developing primordia and their possible crowding within the bud.

Two perspectives on the developmental basis of anisophylly occur, which is the necessary focus for assessing any spatial constraints that might arise from crowding in the developing bud. The first is that the "mode of inception" organizes anisophylly: the leaves are distinctive in size from inception (Dengler 1983*b*). In this view, the primordium of the larger mature leaf is distinguished from the smaller one by a broader zone of initiation and a greater initial size. The concept that differing developmental potentialities of foliar organs are usually expressed at or shortly after initiation, and that accumulation of developmental "capital" in the form of a larger primordium is believed to be an important developmental mechanism in the elaboration of a larger mature leaf (Dengler 1983*a*). Alternatively, the origin of anisophylly may lie in the "mode of expansion": all homologous organs share the same early stages of development and differ in mature form through a process of arrested growth in a primordium at a particular developmental stage and subsequent divergence of ontogenetic pathways (Goebel 1900). Neither of these interpretations considers the role of space within the developing bud on primordial growth and the ultimate expression of anisophylly. Also the alteration of the developmental course of two primordia (opposite each other) on a single node appears to be a unique phenomenon not reported during anisophyllous development.

In this paper, we evaluated the possibility that spatial constraints during bud development and foliar expansion account for anisophylly in *Aucuba japonica* Thunb. This species was selected, because it has decussate phyllotaxis with two leaves developing in close spatial and temporal proximity (Hara 1980). Furthermore, *Aucuba* has a terminal reproductive bud that contains axillary buds (suppressed) and an inflorescence (suppressor). It is easy to identify the inner and outer primordia of each anisophyllous pair in regard to the position of the inflorescence at any stage of development. Thus we were able to describe in detail the features and developmental basis of anisophylly in *Aucuba* and assess whether or not spatial constraints could lead to anisophylly.

Materials and methods

We selected *Aucuba japonica* (Family Cornaceae) plants growing in the Botanical Garden of Kyoto University situated at 35°02′N and 135°47′E and 60 m above sea level. The site is on the Kitashirakawa alluvial fan with sandy soil. Annual mean temperature was 16.1 °C, ranging from a maximum of 36.0 °C in July to a minimum of –5.0 °C in January. Mean annual precipitation was 1539 mm.

Aucuba japonica is an evergreen, woody understory shrub species native to temperate zones of the Himalayas and Japan (Peter et al. 1991) and is widely distributed from warm temperate to cool temperate forests of Japan. In adult stage, shoots are produced through syllepsis (Tomlinson 1978). The minimum age of flowering is 4 years, but reproductive maturity can be delayed as late as 15 years (Kume and Ino 2000). It is dioecious. Phyllotaxis is almost always decussate from vegetative appendages, including bud scales, to the reproductive appendages (Hara 1980). In most instances the shoot bears two to three pairs of foliage leaves.

To measure anisophylly, we selected 10 adult male plants of approximately 20–25 years old. We estimated plant age by counting the number of branches, as branching occurs once a year (Kume and Ino 2000). To compare anisophylly, we selected about 40 sprouts or epicormic shoots, and 45 shoots grew from terminal vegetative buds on those 10 plants. We also measured 40 juveniles grown in pot mostly having three pairs of leaves.

Description of buds

Bud structure is required to understand anisophylly in *Aucuba*. *Aucuba* produces two types of bud: terminal vegetative and terminal reproductive. Juveniles (seedlings) and sprouts produce terminal vegetative buds that extend a single new shoot every year (Kume and Ino 2000) on the supporting shoot apex, until reproductive maturity is attained. Once a plant is mature, succeeding new shoots generally keep flowering in each season, and multiple shoots are extended from the shoot apex. Adult plants produce two types of buds: terminal reproductive and terminal vegetative. The terminal buds (both vegetative and reproductive) commonly have two pairs of bud scales. Axillary buds in the axils of bud scales of the terminal reproductive bud develop into new shoots (Hara 1980), whereas axillary buds in the terminal vegetative bud do not develop. One or both of the axillary buds subtended by the first pair of bud scales may develop into a vegetative shoot. The axillary buds subtended by the second pair of bud scales generally develop into vegetative shoots, although in some instances one or both of them give rise to reproductive shoots. Therefore, a terminal reproductive bud can initiate two to four branches; branches subtended by the second pair of bud scales are ordinarily more vigorous than those of the first pair (Hara 1980). The terminal reproductive bud includes a primordial inflorescence. The terminal vegetative buds are formed on the lower, extremely shaded mature branches (Hara 1980) and are smaller than the terminal reproductive buds. These vegetative buds may begin to flower again, if their size exceeds the critical size (dry weight) for flowering (Kume and Ino 2000).

To describe features of anisophylly and to find a causal relationship, we used various methods to measure the different but inter-related parameters given below:

Measurement of the degree of anisophylly and leaf shape

We expressed the degree of anisophylly as the ratio of leaf area of two leaves opposite each other on a node. For the anisophyllous nodes on an adult shoot (e.g., N_2, N_4 if there were four nodes and if the N_1 pair is isophyllous, since anisophylly is expressed on alternate nodes), we used the ratio of outer (away from the inflorescence) to inner (on the side of the inflorescence) leaf area. For isophyllous nodes (e.g., N_1, N_3 where leaves are grown tangential to the inflorescence), we selected the numerator and the denominator of the ratio for each nodal position randomly because of difficulty in distinguishing the inner or outer leaf. The same principle was followed for shoots from terminal vegetative buds on adult plants, juveniles, and sprouts, since they are all isophyllous. We defined leaf shape (leaf blade narrowness) as the ratio of laminar length to maximum width for all leaf pairs. The same was applied to the leaf primordia.

Measurement of laminar dimensions and area of mature leaves

We measured leaf laminar length and maximum width of fully expanded leaves to the nearest millimetre at each nodal position on 400 shoots selected randomly from around the crown of the 10 sample plants (40 shoots per individual; 20 shoots at the top, and 20 at the bottom of the canopy). "Shoot" was defined as the lateral axis that has grown out in a year from the axillary bud enveloped by the bud scales of the terminal bud. If there were two lateral axes growing from one terminal bud, then the number of shoots for the plant was three including the supporting axis. We also measured fully expanded leaves on sprouts, juveniles, and shoots from terminal vegetative buds on adult plants. Individual leaf area was estimated by the equation $Y = 0.682 \times X$ ($r = 0.999$), where X = lamina length × width. This relationship was established between leaf area (measured by scanning) and lamina dimensions (lamina length × width) of fresh sample leaves ($N = 81$). Lamina length was measured as the distance between the point of attachment to the petiole and the tip of the lamina. Lamina width was measured as the distance between the margins of the lamina at the widest point.

Determination of specific leaf area

To compare specific leaf area (SLA) between groups (described below) of individuals under different light levels as well as between small and large leaves of anisophyllous pairs, we sampled 60 shoots (with two to three pairs of leaves each) from around the crown of the selected individuals (6 shoots × 10 individuals). We measured laminar dimensions of all the leaves. Leaves were oven-dried until reaching constant weight. We estimated specific leaf area as the leaf area per unit dry mass ($\mathrm{cm^2 \cdot g^{-1}}$).

Measurement of bud size

We selected about 300 terminal reproductive buds for measurement on another 300 adult shoots of similar length (30 buds per individual; 15 buds at the top; and 15 buds at the bottom of the canopy). We measured bud length and diameter in November (current season) and at the end of March before bud break in the next year. Buds were rather elliptical (in cross-sectional view), elongated in the direction of the first pair of bud scales, while suppressed in the direction of the second pair. Thus, diameter (a, b and $a > b$) was measured in two directions perpendicular to each other and then the two measurements were averaged.

Assuming a conical shape, we estimated bud size (volume) as follows:

$$S = \frac{1}{3}\pi r^2 h$$

where r is the radius (estimated using $r = D/2$, where $D = (a + b)/2$) and h is bud length. We assumed bud size as the size of the apical dome, where apical dome size represents space available for the growing organs on it. We assumed this because we found a strong correlation between bud size and shoot diameter at the base of the terminal bud, where shoot diameter (reflects the nutritional status of the shoot) represents the size of the subapical region that determines the size and shape of the leaves (Wardlaw 1952).

Measuring light

We measured instantaneous insolation on individual plants (5–10 locations, depending on crown size) using LI-COR sensors (LI-190SA) on an overcast day (mid-August) between 10:30 and 14:00 standard time. Relative photon flux density (RPFD) was estimated in relation to simultaneous photon flux density in the open.

Grouping of sample plants

To compare bud size and the degree of anisophylly in regard to crown light level, we grouped individuals into two. Insolation of individual plants ranged from 0.95% to 8.5% RPFD. Out of 10 sample plants, two subgroups of three individuals were selected. For group one (group I) insolation ranged from about 0.95% to 1.4% RPFD and was treated as dark, while group two (group II) ranged from 2.5% to 8.5% RPFD and was treated as bright. Plants with intermediate insolation were excluded from grouping to make insolation discrete, at least to some extent.

Anatomical observations

For anatomical comparisons in regard to irradiance level, we sampled about 12 terminal reproductive buds from each of the two different individuals having 0.95% and 8.5% RPFD, respectively.

Transverse sectioning of a bud enabled the viewing of all the organs of a bud at a time, their relative positions and tangential extent on the apex, but not their longitudinal appearance. It was particularly necessary for the measurement of primordial width during development. The possibility of cutting the inner and the outer primordia of the potentially anisophyllous pairs, at different height levels, was the least because the lateral buds (e.g., in the axil of the second pair of scales upon which the observations were made) were parallel rather than oblique to the inflorescence axis, especially at the early stages, when inflorescence did not attain much radial growth.

Sampling and preservation of buds, foliage leaves

We collected (10) terminal vegetative buds from juvenile plants and terminal reproductive buds from the top of a single adult crown with 8.5% RPFD (from shoots having two pairs of leaves) for successive analysis. For anatomical comparison, we sampled 10 pairs of anisophyllous leaves (on N_2) and preserved them immediately in 3% glutaraldehyde.

Paraffin embedding of sample, sectioning, and staining

We followed standard methods (Takasoh et al. 1997) for bud and leaf anatomy. Sections of 10-μm thickness were prepared using a rotary microtome. For leaves, a small piece was cut from between the third and fourth lateral vein and in between the mid-rib and the margin of the lamina for sectioning.

Measurement of primordial size

For the buds sampled in the first week of July, we measured primordial length by counting the number of cross-sections in which the structure appeared and then multiplied by section thickness. At this stage, the petiole and lamina were indistinguishable. To measure primordial width, the lowest cross-section was photographed by a camera attached to the microscope. Because of the triangular shape of the primordia, we measured the width of one side by Adobe photoshop and then doubled it.

Measurement of space

The cross-sectional view of the space for axillary bud growth inside the reproductive bud is almost elliptical in shape. The length (distance) of the axes of the ellipse was regarded as a surrogate of the space between the two primordia of a pair (opposite each other). The major axis was assumed to represent greater available space, and the minor axis was assumed to represent scarcity of space. The lengths of the major axis (tangential to inflorescence) and the minor axis (parallel to inflorescence) were measured from the photographs taken for leaf primordial measurements.

Successive measurement of leaf primordial size

To follow the development of anisophylly, we continued measurement of leaf primordial size by destructive sampling from the end of September to early April. To avoid genetic and environmental variation, we sampled reproductive buds from the same crown with 8.5% RPFD and measured primordial length and width to the nearest millimetre under a dissecting microscope from the fresh samples. In these stages, the petiole and lamina were distinguishable in most cases (depending on nodal positions). The lamina was not flat but rather had a triangular configuration. Thus, we measured width of one side of the lamina and then doubled it. To compare anisophylly at bud scale level, we also measured length and width (at the widest point) of bud scales with a caliper.

Excision of inflorescence

To examine if the inflorescence causes anisophylly through spatial constraint, we excised the inflorescence of about 25 terminal reproductive buds on October 15. About 2 months later (December 10), we sampled some of the axillary shoots from the excised buds and measured primordial length and width as described earlier.

Statistical analyses

For variation in bud size, degree of anisophylly, we made comparisons among groups using one-way ANOVA and Mann–Whitney *U* test, and correlation analyses in SPSS Inc. software (Chicago, Illinois).

Results

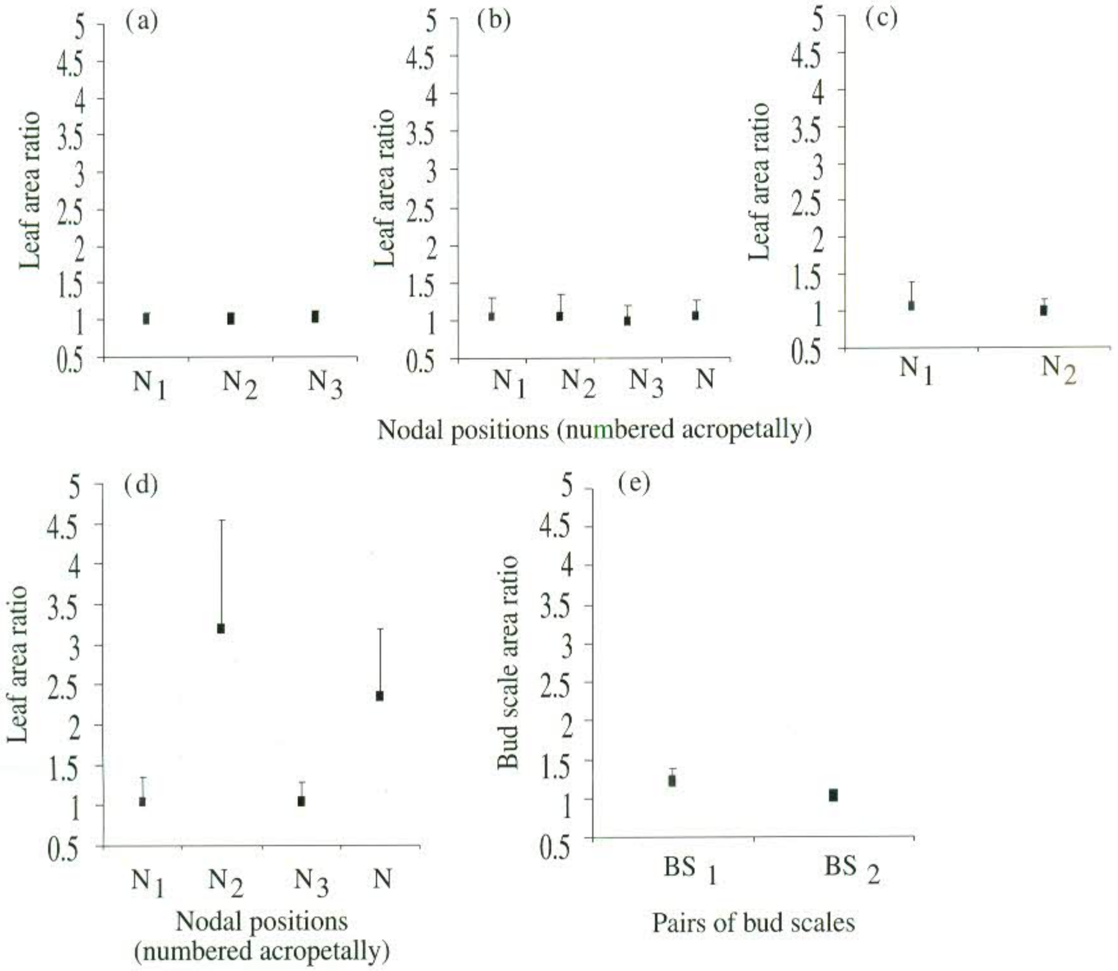
Anisophylly at shoot level

Seedlings or sprouts and (or) epicormic shoots of *Aucuba* did not show anisophylly. Two leaves on any nodal position grew almost equally, and thus the ratio index of anisophylly was close to unity (Figs. 1*a*, 1*b*). Standard deviation (SD) of the ratio was very small for every nodal position on seedlings. For shoots grown from terminal vegetative buds on adult plants, the ratio was also close to unity (Fig. 1*c*), although the SD was a little larger than for juvenile plants. Conversely, shoots from terminal reproductive buds on adult plants showed well-marked anisophylly at alternate nodal positions (Fig. 1*d*). When leaves of a pair (e.g., N_1, N_3) grew tangential to the inflorescence axis, the ratio was close to unity, but when leaf pair orientation changed by 90° (e.g., N_2, N_4), the mean ratio (outer/inner) was significantly greater ($P < 0.001$ Mann–Whitney *U* test) than unity (more than 3 for N_2 and more than 2 for N_4) (Fig. 1*d*). The SD was also greater for N_2 and N_4 pairs, but that of N_1 and N_3 was very small. The mean ratio for N_2 was significantly larger ($P < 0.01$, Mann–Whitney *U* test) compared with N_4. If only the shoots with three pairs of foliage leaves are considered, then the fourth and fifth pairs constituted the first and second pairs of bud scales of the terminal reproductive bud, respectively. The degree of anisophylly of bud scales was reduced greatly, although the first pair remained significantly anisophyllous ($P < 0.001$ Mann–Whitney *U* test) compared with the second pair (Fig. 1*e*). The SD for the first pair was also larger than the second pair of bud scales. Thus, on an adult shoot, the degree of anisophylly was greatest at N_2 and then became reduced towards the tip of the shoot (Figs. 1*d*, 1*e*).

Bud size variation and relation to anisophylly

Size of the terminal reproductive bud was significantly larger under high light intensity for both locations within the crown (Fig. 2*a*) ($P < 0.001$, one-way ANOVA) and between the crowns (Fig. 2*b*) ($P < 0.001$, Mann–Whitney *U* test). The degree of anisophylly at N_2 was inversely related to bud size (Fig. 2*c*). The ratio of two primordia on a node was inversely related to distance (the length of the axes of ellipse on the cross-section of the bud) (Fig. 2*d*). The difference between paired primordia was smaller when they grew tangential to the inflorescence with more space (distance) available between them, and larger when two primordia grew parallel to the inflorescence with smaller space available.

Fig. [illegible] Leaf area ratios (degree of anisophylly) of opposite leaves at the same nodal position. (*a*) Seedling (mid-July). (*b*) Sprout (end of November). (*c*) Shoot from terminal vegetative bud on adult plant. (*d*) Adult reproductive shoot (mid-July). (*e*) Bud scales of reproductive bud (end of March). N, node; subscripts on N indicate position acropetally; BS_1, first pair of bud scales; BS_2, second pair of bud scales. Error bars are SD.



Developmental course of anisophylly

Early stage of development

Foliage primordia of the first pair initiated around late April, and that of the second pair initiated in early July (Fig. 3*a*). At inception, the two primordia of the second pair were different in size, the inner being broad based and more than three times larger than the outer. At this stage, lamina and petiole were indistinguishable.

The inner primordium of the second pair remained larger than the outer one for a longer time (July 16) under high irradiance (Fig. 3*b*), whereas under low irradiance the outer primordium had already attained a larger size than the inner one (July 9) (Fig. 3*c*).

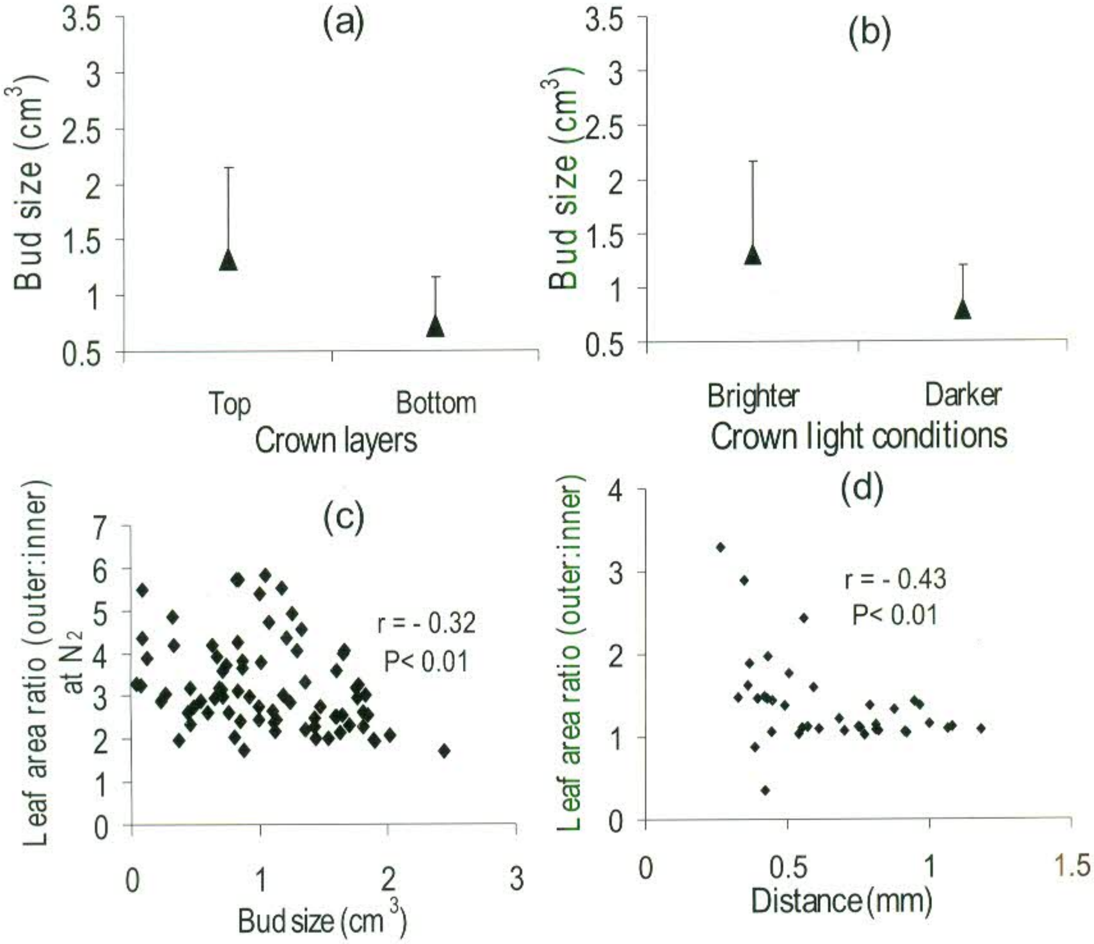
Late stage of development

At the end of October, each primordium was well differentiated into petiole and lamina. Foliage primordia of the N_1 pair that originated tangential to the inflorescence grew similarly and attained almost similar size (Fig. 3*d*). For the second pair, where leaf pair orientation changed by 90°, the outer primordium became larger than the inner one (October 24). At the same time, the lamina of the outer primordium twisted slightly and got inserted into the laminar cover of one primordium of the first pair, whereas the inner primordium remained in the same position and untwisted (Fig. 3*d*).

Development of anisophylly

At inception, the inner primordium of the second pair (N_2) was larger than the outer one (July 2) (Fig. 4*a*). They became almost equal in size around the end of September and continued to grow almost equally for some time and then became different. By the end of October, the developmental course changed, and the outer primordium became larger than the inner one. The outer primordium became significantly larger later in the current season (November 25 to mid-December). Following onset of the next season, the outer primordium became almost twice as large as the inner primordium before bud break in early April of the next year. The degree of anisophylly increased further following bud break and became maximum at full expansion of leaf around the end of June in the next year. Thus, alteration of the ontogenetic course occurred inside the bud, and foliage primordia followed the altered course thereafter until full expansion.

Fig. 2. Size of the terminal reproductive bud and its relation to the degree of anisophylly. (*a*) Within crown bud size variation. Error bars are SD. (*b*) Among the crowns bud size variation. Error bars are SD. (*c*) Relationship between bud size and the degree of anisophylly (mid-July). Note: Only the N_2 position was considered because a higher degree of anisophylly occurred at this nodal position and also because most of the shoots measured had two pairs of leaves, especially under low-light conditions. Bud size was measured before bud break (end of March). (*d*) Relationship between the size difference of a pair of primordia and the distance inside the bud, which is the length of cross-section of the axillary bud (July 2). Data were pooled for N_1 and N_2 positions.



Leaf shape variation during ontogeny of anisophyllous leaves

The outer and the inner leaf primordia of an anisophyllous pair (e.g., N_2) attained proportionally similar laminar dimensional growth during early ontogeny, where growth in longitudinal direction contributed most to primordial size increase (Fig. 4*b*). During the late stage of ontogeny, primordial shape differed significantly, where the inner primordium attained proportionally greater growth in transverse direction compared with the outer primordium. Growth in transverse direction made a greater contribution to size increase in the inner primordium. Following bud burst, the inner leaf primordium attained a sudden greater growth in longitudinal direction, and thereafter the leaf lamina expanded proportionally in both dimensions. The outer leaf primordium attained proportional growth in both dimensions throughout the ontogeny.

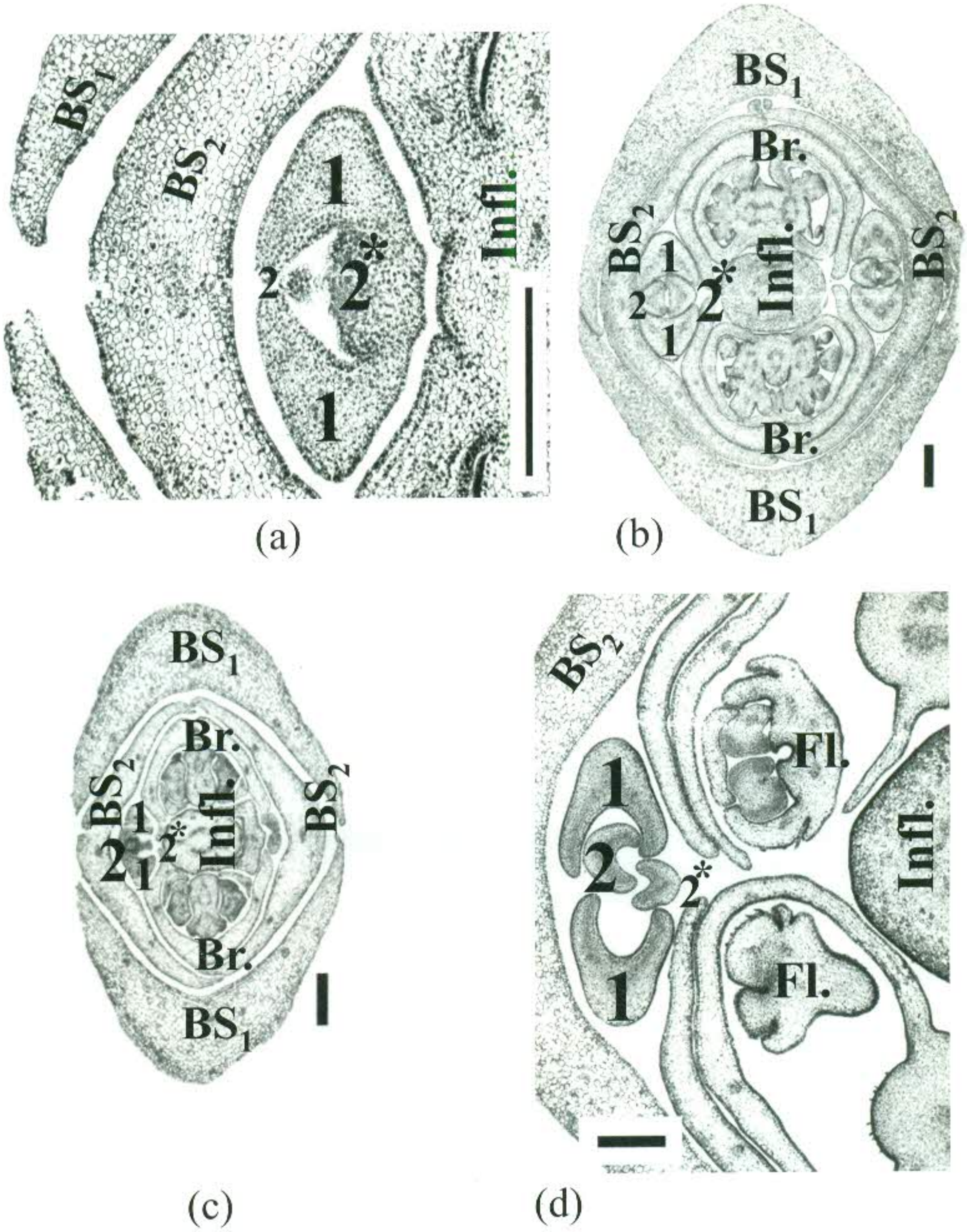
Reduction of anisophylly through inflorescence: Excision experiment

For excised buds, anisophylly reduced significantly ($P <$ 0.001, one-way ANOVA) compared with control. The mean degree of anisophylly on N_2 position was 1.59 for control and 1.1 for treatment, respectively (December 10) (Fig. 4*c*).

Shape variation of mature leaves

Leaf shape varied among epicormic shoot, seedling, and adult shoots, as well as among the nodal positions on a shoot (Figs. 5*a*–5*c*). On epicormic shoots, leaf blade narrowness was greatest, and the lamina became gradually narrower towards the tip of the shoot (Fig. 5*a*). A similar acropetal pattern of blade narrowing was observed for the seedlings (Fig. 5*b*), but the blade was relatively wider than on epicormic shoots for all the comparable nodes. Leaf shape on adult shoots followed a pattern similar to that of seedlings (N_1 and N_3) except the anisophyllous node (N_2) (Fig. 5*c*). On this node, the shape of two leaves was also different ($P < 0.001$, one-way ANOVA). The inner leaf blade elongated less compared with width and was proportionally wider than the outer leaf. The different shape of the N_2 pair (Fig. 5*c*) indicated differential growth constraint among the nodes as well as between the inner and the outer leaf. Thus,

Fig. 3. Developmental course of anisophyllous primordia. Cross-sectional view of terminal reproductive bud. (*a*) Inception of the second pair (July 2); the inner primordium was much larger than the outer one. (*b*) The inner primordium was still larger than the outer one under brighter conditions (July 16). (*c*) The outer primordium of the N_2 pair already had become larger than the inner one under darker conditions (July 9). (*d*) The outer primordium became larger than the inner one (October 24). Lamina of the outer primordium twisted and was inserted into the hollow laminar cover of a primordium of the first pair (1), while the inner one remained in its position. Note: Primordia bearing the same number constitute a pair every time and the font size of the number indicates the primordial size. Asterisks indicate the inner primordium. Infl., inflorescence; Fl., flower primordium; Br., bract; BS, bud scales. Scale bars = 400 μm.



they grew differently and finally became different in shape and size.

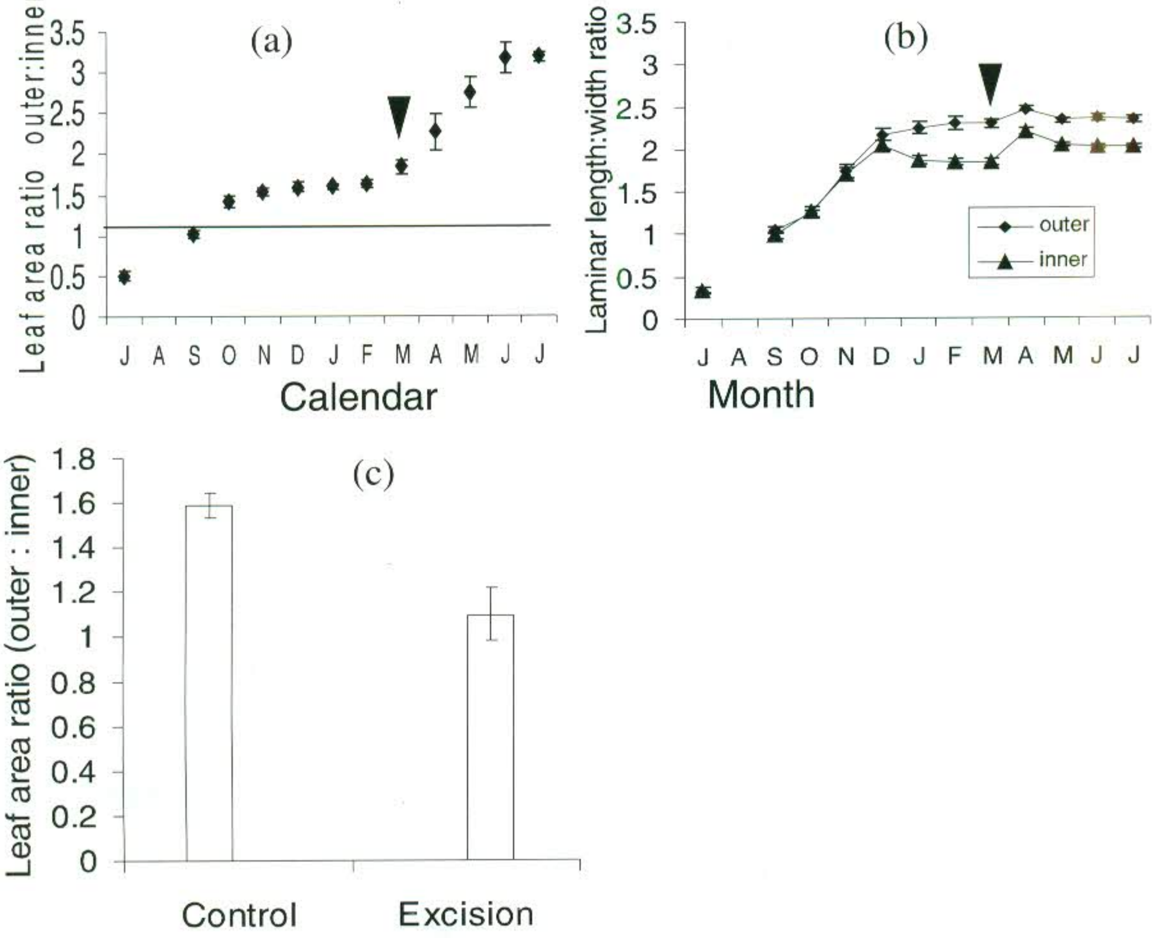
Variation in leaf expansion per unit dry mass

SLA in general was significantly different ($P < 0.01$, one-way ANOVA) under different light levels (140.69 ± 19.38 SD and 133.75 ± 21.39 SD for groups I and II, respectively) (Fig. 5*d*), but no significant difference ($P > 0.05$, one-way ANOVA) between large and small leaves of anisophyllous pairs was detected (Fig. 5*e*).

Histology of anisophyllous mature leaves

Anisophyllous leaves did not differ at maturity in cell shape nor size or number of cell layers (Figs. 6*a*, 6*b*). For both leaves, total layers of cells varied from 10 to 12, including the epidermal layers. The single layered epidermis

Fig. 4. Ontogenetic course of anisophylly (*a*) and leaf shape variation (*b*). The N_2 position was taken for a demonstration of anisophylly and leaf shape variation in *Aucuba*, since the degree of anisophylly was greatest at N_2. Note: Arrowheads indicate differences just before bud burst. (*c*) Reduction of anisophylly on the N_2 position by excision of inflorescence (December 10).



was covered with a thick cuticle layer mainly on the adaxial side. Stomata were confined to the abaxial epidermis. Chlorenchyma tissue was composed of two layers of large, rectangular cells (in surface view). Chloroplasts were mainly concentrated on adaxial side. Spongy parenchyma tissue was composed of round, elongated cells suppressed at both ends.

Anatomical comparison of terminal vegetative and reproductive buds

Each bud type has two pairs of bud scales, and each bud scale is covered by the other at one end. The vegetative bud had three pairs of foliage primordia, where two consecutive pairs were arranged decussately around the shoot axis (Fig. 7*a*). Primordia of each pair were identical to each other. The two outermost primordia constituted the first pair, and the innermost primordia formed the latest pair. The longitudinal section of the terminal vegetative bud (Fig. 7*b*) had three pairs of foliage primordia with similar space availability for both the primordia of each pair. In the reproductive bud (Figs. 7*c*, 7*d*), most of the space was occupied by the inflorescence and bracts. The passage for axillary bud growth was constricted (large arrowhead) between the inflorescence and the second bud scale (Fig. 7*d*).

Discussion

Place-dependent suppression of growth plays an important role in plant morphogenesis and phylogeny through modulation of size and shape of organs (Basile and Basile 1993). Growth suppression might occur for various reasons, e.g., chemoregulatory systems where auxin plays a key role (Basile and Basile 1993) or simply through spatial constraint. Anisophylly in *Aucuba* resulted from place-dependent growth suppression along the axillary shoot where space limitation seemed to play the key role. Foliage primordia that originated tangentially to the inflorescence seemed to have greater and indentical space and, thus, were able to follow a similar ontogenetic pathway (White 1957). As a result, they became isophyllous. Change in leaf pair orientation from tangential to parallel to the inflorescence axis increased space limitation for the parallel pair and showed well-marked anisophylly. Thus, nodes of an adult shoot ranged from isophyllous to well-marked anisophyllous (Sánchez-Burgos and Dengler 1988) depending on the spatial condition during foliage development. Acropetal reduction in the degree of anisophylly on the axillary shoots appeared to be related to spatiotemporal change in space availability within the bud (Hara 1980).

Fig. .. Variation in the shape of fully expanded leaves that developed from different bud types. Leaf blade narrowness was measured on epicormic shoots (*a*), seedlings (*b*), and adult shoots (*c*). Patterns were similiar to that of seedlings, except for the N_2 position where the lamina of the inner leaf elongated less relative to its width. Note: In each case, except the N_2 position on the adult shoot, nodal positions on different shoot types represent the shape of both leaves on it, as their shape was almost identical. (*d*) Specific leaf area (SLA) under different light levels (data pooled for leaves from all nodal positions on a shoot). (*e*) SLA of anisophyllous leaves on a node (N_2). Note: Data were pooled for all the individuals.

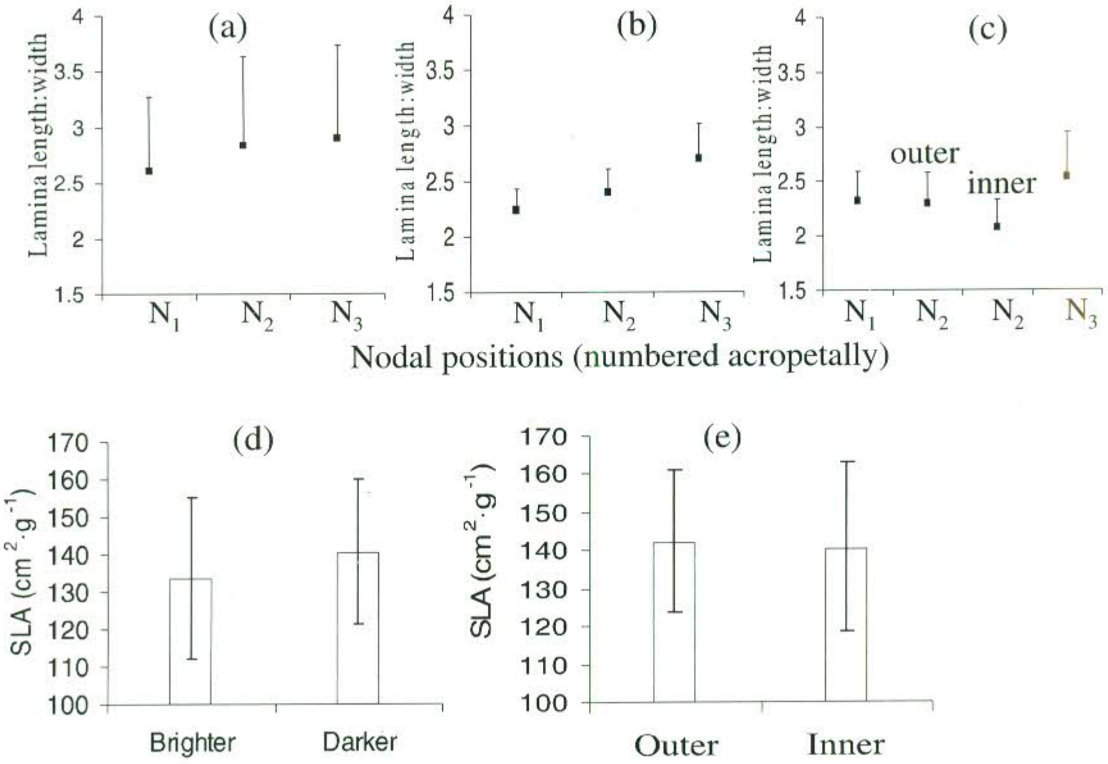


Fig. 6. Anatomy of fully expanded anisophyllous leaves at the N_2 position. (*a*) Cross-section of outer leaf. (*b*) Cross-section of inner leaf. Scale bar = 100 μm.

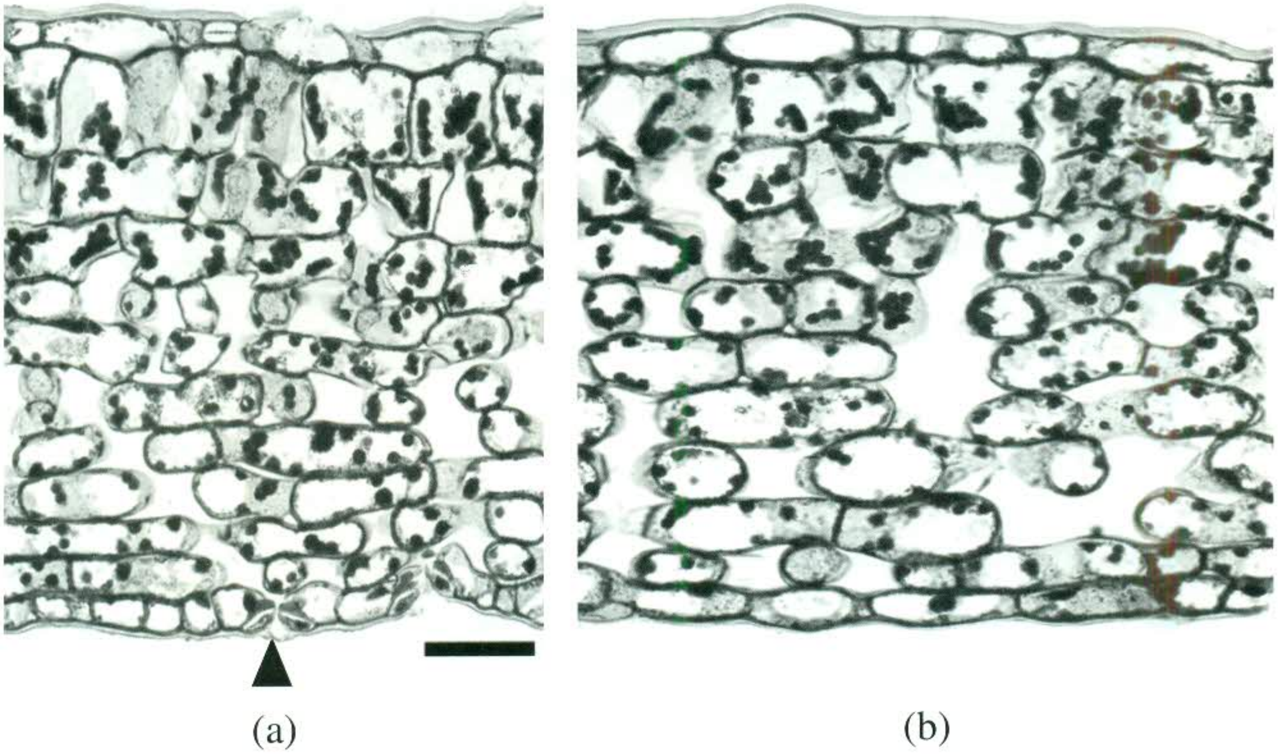
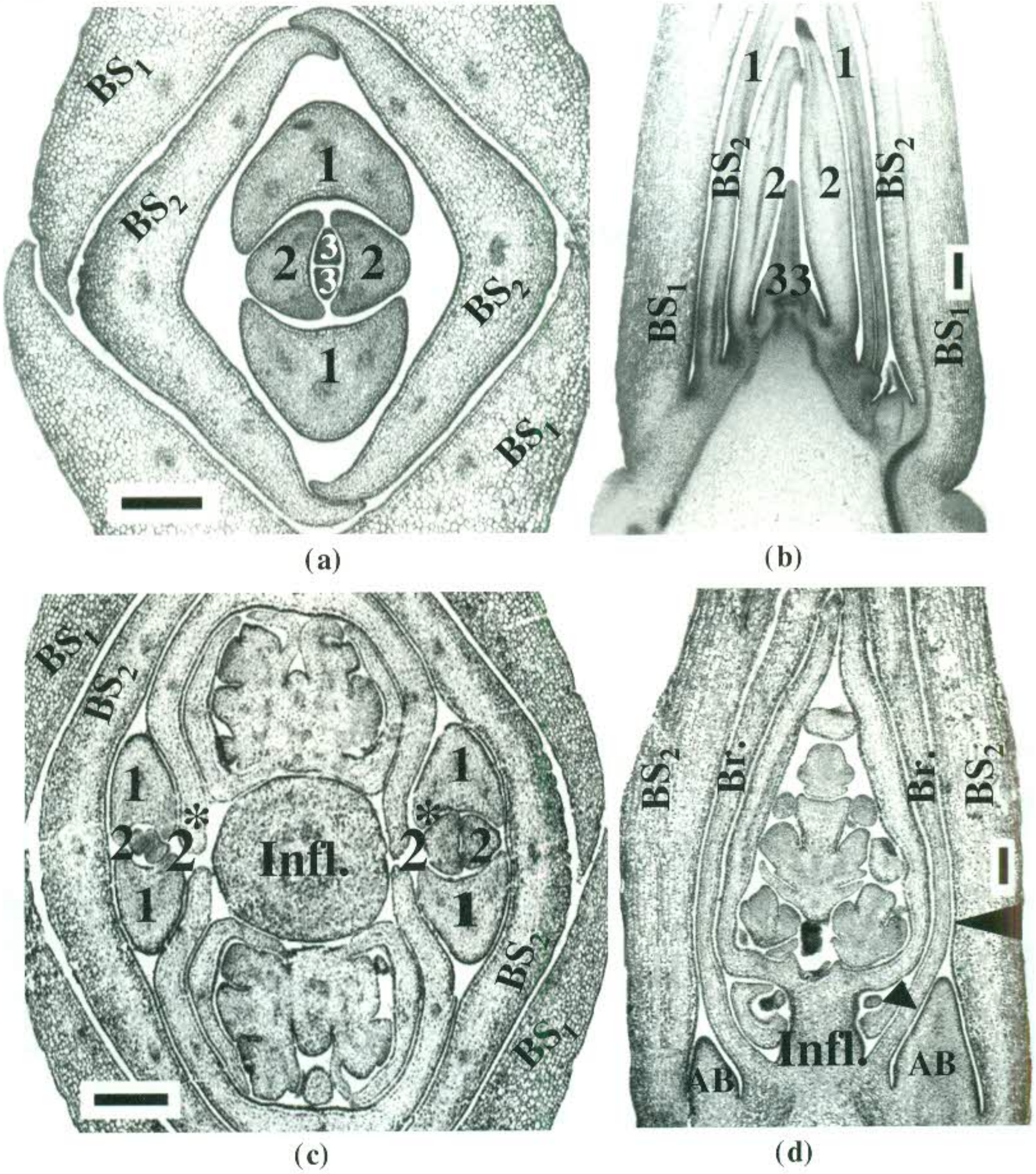


Fig. 7. Leaf arrangement and comparison of bud internal morphology. Note: Primordia bearing the same number constitute a pair every time and the font size of the number indicates the primordial size. Asterisks indicate the inner primordium. Infl., inflorescence; Br., bract; BS, bud scales; AB, axillary buds. (*a*) Cross-section of a terminal vegetative bud with primordia arranged in a decussate manner (October 24). Vacant space around the primordia indicates their loose packing in the bud. (*b*) Longitudinal section of a terminal vegetative bud shows three pairs of foliage primordia. Exceptionally a lateral bud growth was observable (right side) in the axil of BS_1 (October 24). (*c*) Cross-section of a terminal reproductive bud. (*d*) Longitudinal section of a terminal reproductive bud shows two axillary buds in the axil of BS_2 (July 2). Organs are tightly packed, and most of the space in the bud is occupied by the inflorescence. Note: Large arrowhead indicates the constricted passage that might exert constraint during elongation growth of AB. Small arrowhead shows outer wall of the inflorescence from where the inner primordium of the N_2 pair would get more direct obstacle during elongation of AB. Scale bars = 400 µm.



Acropetal reduction in the degree of anisophylly (Figs. 1*d*, 1*e*) might be partly due to delayed initiation as well as premature termination of growth of the uppermost leaves on the shoot (White 1957). Anisophylly disappeared toward the end of the growing season and renewed following bud break in the next spring. Fuller (1913) found a similar trend in species of *Sempervivum*. All the above phenomena indicated that two leaves of a pair could grow equally if they were provided with equal spatial opportunity as in the case of epicormic shoots, seedlings, and shoots, which grew from terminal vegetative buds on adult plants (Figs. 1*a*, 1*b*, 1*c*).

The degree of anisophylly also varied depending on environmental conditions, e.g., light regime through affecting shoot vigor (Boshart 1912, as mentioned by Fuller 1913) indicating a possible role of plant nutrition. A possible nutritional effect was also reflected through bud size i.e., shoot apical size (Wardlaw 1952; Allsop 1967) under different light conditions (Figs. 2*a*, 2*b*) that also provided space for leaf growth, at least radially.

Despite a pronounced effect of light level on leaf expansion per unit dry mass i.e., SLA, anisophyllous leaves did not differ in this regard, which indicated that the expansion of both leaves of a pair might be affected equally (Dengler and Sánchez-Burgos 1988) without affecting the degree of anisophylly. In that case, expression of increased degree of anisophylly under low irradiance might be due to nutritional status of the shoot, which in turn might be dependent on irradiance level, as mentioned earlier (Wardlaw 1952; Dengler and Sánchez-Burgos 1988), or by shade-induced crowding (Givnish 1984), as discussed later, or by both.

Together with apical size, foliage distribution on the shoot seemed to affect the degree of anisophylly. The prerequisite for the development of anisophylly is suppression of growth on one side and enhancement on the other side (Dengler 1999). Thus, depending on space availability, the degree of anisophylly depends on how early and to what extent the suppression occurs. Smaller bud size indicated significantly limited space for growing organs on the shoot apical dome under low irradiance. Shade-induced crowding (Givnish 1984) also caused congestion of the growing space for primordia (Wiehler 1978). Thus, under low irradiance, an inner primordium might experience suppression rather earlier and to a greater extent compared with high irradiance conditions. Therefore, the outer primordium exceeded the growth of the inner primordium earlier under darker conditions than under the brighter conditions that promoted anisophylly.

Developmental course of anisophylly

Although Hara (1995) reported that the initial unbalanced size was maintained until the immediate postinitiation stage and then the outer primordium began to grow faster, our investigation revealed that the inner primordium remained larger for a longer time. It took nearly two plastochron index units (the plastochron index of *Aucuba* is 1.5 months (Hara 1995)) for the primordia to attain equal size. This also indicated that the changes in ontogenetic courses were rather gradual, where the growth of the inner and the outer primordia became constrained gradually and differently, and this caused alteration of the developmental course.

Despite a larger initial size and thus greater developmental potential (Sánchez-Burgos and Dengler 1988), the inner primordium did not produce a larger leaf. So it is evident that primordial size at inception is not a prerequisite for expressing anisophylly. Instead, the ontogenetic course of development had a more significant impact on final leaf size. Therefore, the concept of accumulation of developmental "capital" in the form of a larger primordium (Dengler 1983*a*) could not be generalized. The outer primordium that had already attained a larger size (Fig. 3*d*), because of its weaker base (anatomical observation), easily twisted and got inserted into the hollow laminar cover of a primordium of the first pair. This might enable the outer primordium to bypass growth suppression (at least partly) from the enlarging inflorescence. Because of the longitudinal hollow passage within the laminar cover of the sheltering primordium, the outer primordium could elongate rather easily. Successive anatomical observation indicated that such a sheltering occurs rather later in the season, when further growth of the inflorescence created increased stress. Conversely, the inner primordium, because of its thicker and stronger base, could not be twisted, but rather maintained a straight form with its position unchanged. Thus, it could not avoid growth suppression from the enlarging inflorescence. It is evident that an initially smaller primordium could result in a larger final size, if the opportunity existed. Thus, growth opportunity rather than primordial size appeared to be more important for induction of anisophylly in *Aucuba*.

Spatial constraint likely to be a factor for growth suppression in *Aucuba*

Comparison of leaf blade narrowness on seedling, adult shoot, and epicormic shoot (grown from a dormant bud without bud scales) (Figs. 5*a*–5*c*) indicated that bud scales could exert some formative effect (possibly mechanical) on the shape of leaf blade. Acropetal increase in leaf blade narrowness (Figs. 5*a*–5*c*), a common phenomenon to many plant species, also provides an example of growth retardation due to spatial constraint; because, on the shoot apex, later formed inner primordia encompassed by the earlier formed outer primordia (Figs. 7*a*–7*c*) suffer from greater space limitation, at least, radially. Changes in lateral stem symmetry from an initial dorsiventrally flattened, elliptical shape at the base to an almost round shape near the tip (Hara 1980, 1997) pointed that the basal part of the lateral shoot was under greater compression, and that compression was reduced towards the tip. Dorsiventrality indicated that tissue (shoot) extension followed the direction of less constraint. The inner leaf of N_2 followed the same principle of tissue extension, where the leaf blade widened tangentially relative to elongation growth (Fig. 5*c*). The elongation growth of the inner leaf was highly suppressed compared with that of the outer leaf (Larson and Richards 1981). Growth in longitudinal direction of the inner primordium was greatly suppressed in the later stage of ontogeny, when the inflorescence grew much larger (Fig. 4*b*). Such suppression retarded primordial growth without affecting leaf histogenesis. Anatomical observations indicated that anisophyllous leaves did not differ at maturity, and both leaves underwent normal histogenesis despite suppression (Sánchez-Burgos and Dengler 1988).

White's (1955) assumption of spatial constraint responsible for anisophylly in *Acer pseudoplatanus* L. was not clarified by evidence. Also the effect of a collar formation around the shoot apex (by the fusion of lateral margins of the first pair of primordia) that was said to have a significant impact on space was unclear. Both the primordia of the second pair might have a similar space limitation, if they initiate after completion of the collar formation. But if they initiate prior to completion of the collar formation, then the primordium on the side of the main axis would have a greater space at the initial stage, as the collar formation is delayed on this side (White 1955), and thus should be larger. But it is always the primordium adjacent to the main axis that is smaller than the other one (White 1955). Contrarily, for *Aucuba* the primordium adjacent to the inflorescence is always larger than the outer one initially. Anatomical observation suggested that delayed completion of lamina formation of the first pair of foliage primordia on the side of the outer primordium might allow greater space for it to grow larger, and the reverse was true for the inner primordium.

Comparison of bud internal morphology (Figs. 3, 7) clearly indicated that spatial constraint caused by the inflorescence induced anisophylly. Isophyllous epicormic shoots and shoots from terminal vegetative buds in adult crowns showed that anisophyllous leaf production switched over to isophyllous development, as the constraint (absence of inflorescence) was lifted (Dengler 1999). Thus, physical constraint was most likely to induce anisophylly, where primordial growth (e.g., inner) was suppressed by the growing inflorescence. Sudden greater longitudinal growth of the inner primordium following bud burst perhaps indicates a release from suppression by the inflorescence (Fig. 4*b*). Also the result of excision of the inflorescence (Fig. 4*c*) (although leaves did not get enough time for full expression of anisophylly) indicated that anisophylly can be removed, if the constraint is withdrawn in time, and it seemed unlikely to induce anisophylly by the physical properties of bud scales.

Conclusion

Anisophylly and reproductive maturity in *Aucuba* are concomitant events, and a causal relationship between them was shown. Identical spatial condition for both leaf primordia of a pair on any nodal position resulted in isophylly at juvenile phase of *Aucuba*, on sprouts or epicormic shoots and shoots from terminal vegetative buds on adult plants. Evidence showed that mechanical stress only could retard primordial growth, where the stress was created by a growing inflorescence that reduced the space for axillary bud growth.

Primordial size was not the precursor of final leaf size, and thus, final leaf size was not predictable from primordial size. Mode of expansion rather than mode of inception was relatively more important in expressing anisophylly. None of the previous interpretations regarding anisophylly did entirely fit *Aucuba*. Rather our results showed a third alternative developmental process of anisophylly, where initially smaller primordia became larger in the course of development and vice versa.

Acknowledgements

We thank the authority of the Botanical Garden, Kyoto University, for allowing us to conduct our investigation in the garden. We also express our deep gratitude to Dr. Minoru Fujita for allowing us to use his laboratory facility and to Dr. Naofumi Nomura for his help in conducting anatomical work. We are also thankful to Dr. Martin J. Lechowicz of McGill University, Canada, for reviewing the manuscript and providing us with helpful suggestions.

References

Allsop, A. 1967. Heteroblastic development in vascular plants. Adv. Morphogen. **6**: 27–171.

Basile, D.V., and Basile, M.R. 1993. The role and control of the place dependent suppression of cell division in plant morphogenesis and phylogeny. Memoirs Torrey Bot. Club, **25**: 63–84.

Dengler, N.G. 1980. The histological basis of leaf dimorphism in *Selaginella martensii*. Can. J. Bot. **58**: 1225–1234.

Dengler, N.G. 1983*a*. The developmental basis of anisophylly in *Selaginella martensii* I. Initiation and morpholoyg of growth. Am. J. Bot. **70**: 181–192.

Dengler, N.G. 1983*b*. The developmental basis of anisophylly in *Selaginella martensii* II. Histogenesis. Am. J. Bot. **70**: 193–206.

Dengler, N.G. 1999. Anisophylly and dorsiventral shoot symmetry. Int. J. Plant Sci. **160**(Suppl. 6): S67–S80.

Dengler, N.G., and Sánchez-Burgos, A.A. 1988. Effect of light level on the expression of anisophylly in *Paradrymonia ciliosa* (Gesneraceae). Bot. Gaz. **149**: 158–165.

Dostal, R. 1967. On integration in plants. Harvard University Press, Cambridge.

Fuller, G.D. 1913. Anisophylly. Bot. Gaz. **55**: 471.

Givnish, T.J. 1984. Leaf and canopy adaptations in tropical forests. Physiological ecology of plants of the wet tropics. *Edited by* E. Medina, H.A. Mooney, and C. Vazquez-Yanes. Junk, The Hague. pp. 51–84.

Goebel, K. 1900. Organography of plants. English edition. Oxford University Press, New York.

Hara, N. 1980. Shoot development of *Aucuba japonica* I. Morphological study. Bot. Mag. Tokyo, **93**: 101–116.

Hara, N. 1995. Shoot development of *Aucuba japonica* III. Shoot apex at the stage of initiation of the polage leaf. Phytomorphology, **45**: 229–237.

Hara, N. 1997. Shoot development of *Aucuba japonica* IV. The shoot apex at the late stage of first growing season. Phytomorphology, **47**: 437–444.

Kume, A., and Ino, Y. 2000. Differences in shoot size and allometry between two evergreen broad–leaved shrubs, *Aucuba japonica* varieties in tow contrasting snowfall habitats. J. Plant Res. **113**: 353–363.

Larson, P.R., and Richards, J.H. 1981. Lateral branch vascularization: its circularity and its relation to anisophylly. Can. J. Bot. **59**: 2577–2591.

Mueller, P.A., and Dengler, N.G. 1984. Leaf development in the anisophyllous shoots of *Pellionia daveauana* (Urticaceae). Can. J. Bot. **62**: 1158–1170.

Peter, C.A., Gary, W.K., and Jeffreu, G.N. 1991. Light intensity influences growth and leaf physiology of *Aucuba japonica* 'Variegata'. Hortscience, **26**: 1485–1488.

Sánchez-Burgos, A.A., and Dengler, N.G. 1988. Leaf develoment in isophyllous and facultatively anisophyllous species of Pentadenia (Gesneriaceae). Am. J. Bot. **75**: 1472–1484.

Sinnott, E.W., and Durham, G.B. 1923. A quantitative study of anisophylly in *Acer.* Am. J. Bot. **10**: 278–287.

Takasoh, T., Tobe, T., and Aida, M. 1997. Method of sectioning for microscopic observation. Plant cell technology series 9: Protocols for observing plant cells. *Edited by* H. Fukuda, M. Nishimura, and K. Nakamura. Published by SHUJUNSHA Co. Ltd., Tokyo. pp. 20–33. [In Japanese.]

Tomlinson, P.B. 1978. Branching and axis differentiation in tropical trees. *In* Tropical trees as living systems. *Edited by* P.B. Tomlinson and M.H. Zimmermann. Cambridge University Press, Cambridge, London. pp. 187–207.

Wardlaw, C.W. 1952. Experimental and analytical studies of Pteridophytes XVIII. The nutritional status of apex and morphogenesis. Ann. Bot. (London), **62**: 207–218.

White, D.J.B. 1955. The architecture of the stem apex and the origin and development of the axillary buds in seedlings of *Acer pseudoplatanus* L. Ann. Bot. N.S. Vol. XIX **No. 75**: 437–449.

White, D.J.B. 1957. Anisophylly of lateral shoots. Ann. Bot. N.S. Vol. XXI **No. 82**: 249–255.

Wiehler, H. 1978. The genera *Episcia*, *Alsobia*, *Nautilocalyx*, and *Paradrymonia* (Gesneriaceae). Selbyana, **5**: 11–60.