

MERISTEM FATE AND BULBIL FORMATION IN *TITANOTRICHUM* (GESNERIACEAE)¹

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Titanotrichum oldhamii (a monotypic genus from Taiwan, Okinawa, and adjacent regions of China) has inflorescences bearing either showy yellow flowers or asexual bulbils. Asexual reproduction by bulbils is important in natural populations, and bulbil production increases in August and September at the end of the flowering season (which runs from June to the end of September). The bulbils are small (~1–2.5 mm long) and numerous. They consist of a small portion of stem (bract-stem) topped by opposite storage bracts that enclose a minute apical meristem. A secondary root develops from the side of the bract-stem. The floral meristem of *T. oldhamii* has three possible fates: (1) bulbil formation, (2) flower formation, or (3) bracteose proliferation. Bracteose proliferation rarely occurs and appears to be a developmental transition between the bulbiliferous and racemose inflorescence forms. It is strongly reminiscent of the *floricaula* and *squamosa* mutants of *Antirrhinum*. In the bulbiliferous form a single floral primordium, which would normally produce one flower, gives rise to ~50–70 bulbils by repeated subdivision of the meristem. This form of bulbil production appears to be unique to *Titanotrichum*. Occasionally a floral meristem divides, but the subdivision forms multiflowered units of up to four flowers rather than bulbils, suggesting that meristem fate is reversible up to the first or second meristem subdivision. In *Titanotrichum*, therefore, primordium fate is apparently not determined at inception but becomes irreversibly determined shortly after the appearance of developmental characteristics of the floral or bulbil pathway.

Key words: bracteose proliferation; bulbil; China; floral meristem; *floricaula*; gemmae; Gesneriaceae; Japan; *squamosa*; Taiwan; *Titanotrichum oldhamii*; vivipary.

Titanotrichum oldhamii Soler. (Gesneriaceae) was first discovered in 1864 by Richard Oldham, collecting for the Royal Botanic Gardens Kew, during his trip to Formosa (now Taiwan) (Hemsley, 1890, 1895; Solereder, 1909; Sealy, 1949). *Titanotrichum oldhamii* was cultivated in Europe as an ornamental for its distinctively deep yellow corolla tube with lobes blotched dark crimson-brown. The inflorescence of *T. oldhamii* is an indeterminate raceme, although the family Gesneriaceae, to which it belongs, usually produces pair-flower cymes (Weber, 1973, 1978, 1982, 1995; Pan et al., 2002).

It grows in shaded habitats along creeks, particularly on dripping cliffs or moist limestone slopes in dense forest and on rocks near caves and waterfalls. It is not a common plant but has a scattered distribution in Taiwan, the Fujien province of China, and the Ryukyu Islands of Japan (Henry, 1898; Hayata, 1908, 1911; Hemsley, 1909; Walker, 1976; Wang et al., 1998). Interestingly, although it produces many flowers in the wild, it rarely sets seed and appears to rely largely on asexual reproduction by bulbils and rhizomes. Genetic variation and

the failure of seed set are being studied at Edinburgh (C.-N. Wang, unpublished data).

Gesneriaceae species are known for their great range of morphological variation, resulting from variation in meristem behavior (Jong and Burtt, 1975; Möller and Cronk, 2001). While in most Gesneriaceae the unusual meristem behavior affects only vegetative parts (Burtt, 1970; Tsukaya, 1997; Imaichi et al., 2000), *Titanotrichum* is unusual in having variable meristem behavior in reproductive parts.

There are many plants in which all or some flowers of an inflorescence are converted into asexual bulbils. In most cases, a single floral meristem is replaced by a single bulbil (e.g., *Polygonum viviparum* L., *Ranunculus ficaria* L., *Saxifraga cernua* L., *Allium* spp., *Festuca vivipara* (Rosenv.) E. B. Alexeev.; Kerner, 1904; Troll, 1964; Engell, 1973; Arizaga and Ezcurra, 1995; Briggs and Walters, 1997; Diggle, 1997). In *Titanotrichum* however, a single floral meristem is replaced by a cluster of ~50–70 bulbils (Stapf, 1911; Hayata, 1912). In *Mimulus gemmiparus* W. A. Weber, it is not the floral meristem but the adjacent proximal meristem (dormant in other species of *Mimulus*) that develops into a bulbil (Weber, 1972; Moody et al., 1999). Pseudovivipary, often found in alpine or arctic habitats, is often assumed to be an adaptation to poor sexual reproduction under extreme conditions (Kerner, 1904; Youngner, 1960). In contrast, *Titanotrichum* grows in a subtropical area with a favorable environment, although sometimes in deep shade.

As part of a wider study of reproduction in this species, the morphology of bulbil development was investigated. *Titanotrichum* is unique in producing large numbers of bulbils in place of a single flower. We were therefore interested in answering the following questions: (1) how a single floral meristem is replaced with numerous bulbil meristems, (2) how an inflorescence changes from flower to bulbil production, and (3) how bulbils differ from seeds as functional reproductive units.

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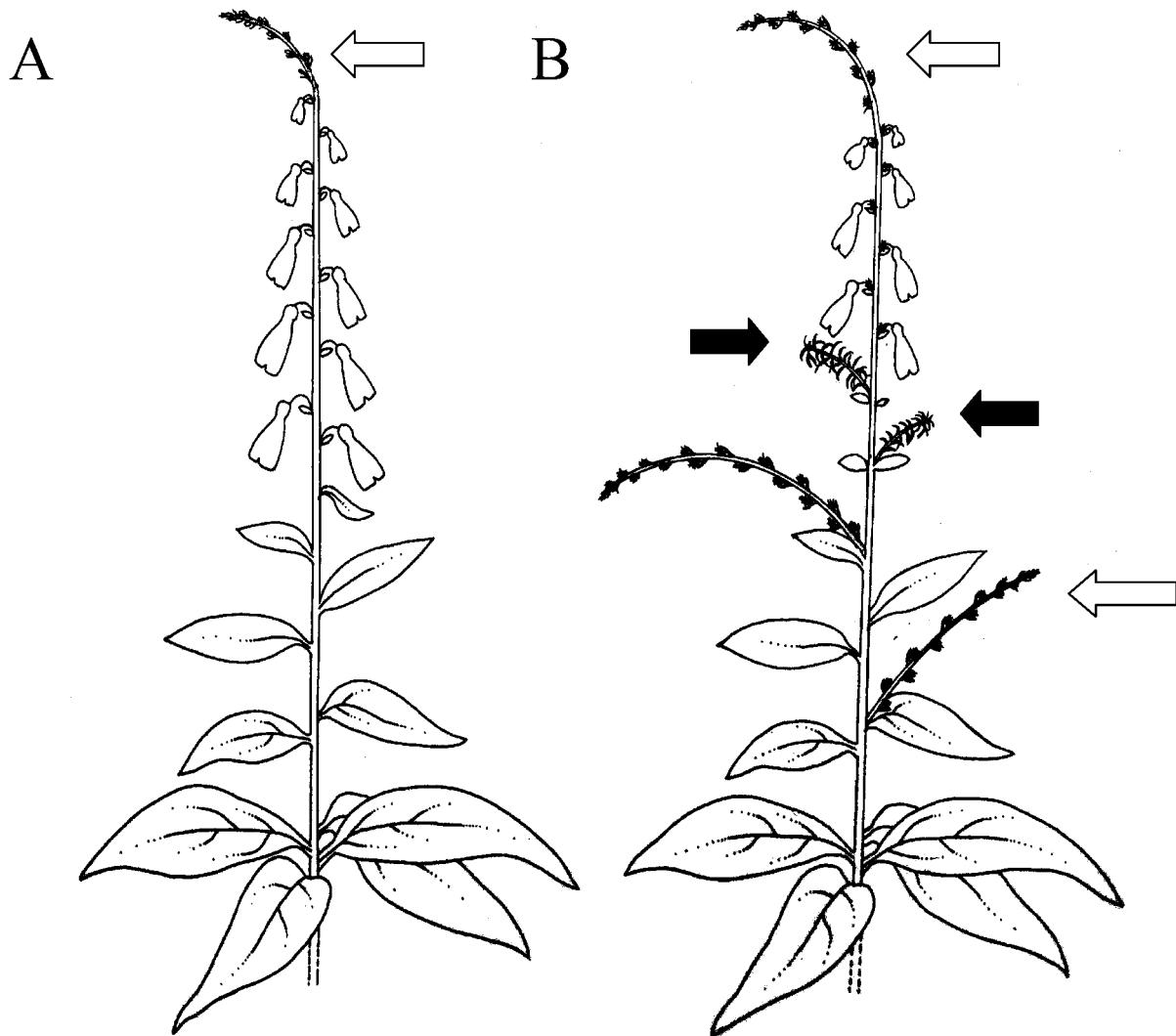


Fig. 1. Plant architecture in natural populations of *Titanotrichum oldhamii*. Growth forms differ in summer with long daylength (A) and autumn with short daylength (B). In (B), as daylength declines, bulbiliferous clusters and bracteose and inflorescence branches are produced eventually at top of the inflorescence and also in the axils of leaves and flowers.

MATERIALS AND METHODS

Source of plant material and growth conditions—We investigated 26 natural populations of *Titanotrichum* from Taiwan, China, and the Okinawa Islands of Japan during the summer of 1999 and 2000. The 145 accessions of living material collected from these locations were first cultivated at the Institute of Botany, Academia Sinica, Taipei, and then moved to the Royal Botanic Garden, Edinburgh, where they were grown between 18°C (night) and 20°C (day) in a controlled climate glasshouse.

Because *Titanotrichum* rarely produces seed, mature seeds were obtained from a pollination experiment in Yangmingshan National Park (25°09' N, 121°33' E), Taipei region, during the summer of 1999. Seed germination requires light and was carried out under constant lighting in petri dishes at 22°C and 90% relative humidity in controlled environment growth cabinets, on wet filter paper. Growth experiments were conducted in walk-in growth rooms manufactured by Swann Technology (Royston, UK) with controllable temperature and daylength. Lighting was provided by 13-W cool white fluorescent tubes.

Fixation of plant tissue—Different stages of the inflorescence shoot, seedlings, and germinating bulbils were fixed overnight in FAA (18 parts of 70% ethanol : 1 part glacial acetic acid : 1 part formalin) and taken through an

ethanol series to 100% acetone dehydration before proceeding to critical point drying (CPD) with an Emitech K850 machine (Ashford, UK). The dried samples were immediately mounted on aluminium stubs using carbon discs and coated twice with gold palladium for 2 min (from different angles) in an Emscope SC500 sputter coater (Quorum Technologies, Newhaven, UK). To see the development of the meristem clearly, most bracts and bracteoles were removed, especially in the young inflorescence, using fine forceps either before fixation or, less satisfactorily, after CPD.

Scanning electron microscopy—Specimens were examined with a Zeiss DSM scanning electron microscope (Oberkochen, Germany) at a working distance of between 9 and 14 mm and an accelerating voltage of 5 kV.

RESULTS

Distribution of flowers and bulbils on inflorescences, in individuals, and in populations—The inflorescence of *Titanotrichum oldhamii* is basically racemose, with spirally arranged bracts, each subtending a single axillary flower with two bracteoles (Figs. 1, 2A). Occasionally inflorescence branches or multiflowered units of up to four flowers (each

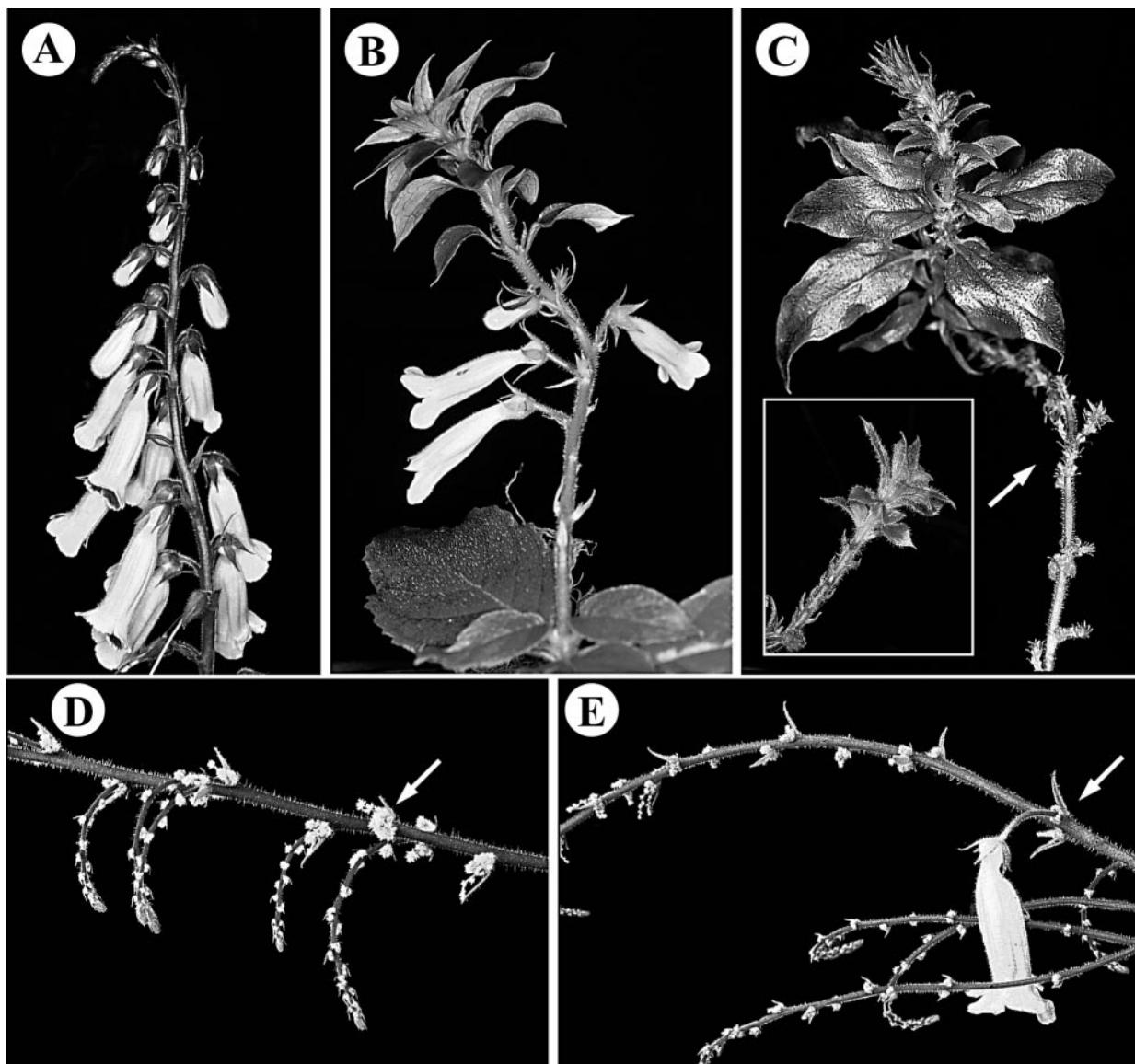


Fig. 2. *Titanotrichum oldhamii*. (A) Normal inflorescence with bulbils beginning to initiate at the tip of the inflorescence. (B) The reversion of flowers to leaves (bracts). (C) Bracteose proliferation in the lower part of the inflorescence. Inset at 5 \times magnification. (D) Bulbil inflorescence with branching. (E) Bulbil clusters replace flowers in upper part of the inflorescence and at flower axils. Arrows in D and E indicate the position of bulbil clustering.

with two bracteoles) may arise at some nodes (from serial multiple buds in the axil of the same bract). *Titanotrichum* produces normal racemose inflorescences of this type during the summer, but at the end of the season (i.e., the end of August), the upper part of the raceme starts to convert to the production of bulbils. Bulbil clusters, bulbil clusters around flowers, or new bulbil-bearing inflorescence branches are formed in the axils of the bracts in the place of single flowers.

Three different types of inflorescence can be seen at this stage: (1) bulbils and flowers mixed, (2) bulbils only, and (3) bracteose proliferation (consisting of repeated production of bracts rather than flowers or bulbils) with some flowers (Fig. 2B–C). In addition, bulbil clusters also proliferate in the axils of existing flowers in the lower part of the inflorescence (Fig. 2D). Within 2 or 3 wk, tens of thousands of bulbils may fall from one individual and are ready to grow. Production of different inflorescence types appears to be under environmental

rather than genetic control, because it may vary on the same individual from year to year. Most individuals, both in the field and glasshouse, start with flowering inflorescences but end up converting to bulbil production and initiating new bulbil inflorescences. Juvenile plants (i.e., ~1 yr old) growing in dense shade are an exception, as they produce only bulbils during the flowering season.

Development of the inflorescence—*Titanotrichum oldhamii* is a persistent rhizomatous herb. Several shoots can arise from the rhizome, and during early vegetative growth, a pair of two unequal leaves is produced at each node, resulting in a decussate phyllotaxy typical of the Gesneriaceae and other Lamiales such as *Antirrhinum* (Carpenter et al., 1995; Vincent et al., 1995). When it enters the reproductive phase, it converts to spiral phyllotaxy, in which a single “floral” meristem initiates at each node, with a single bract. In most related plants, such

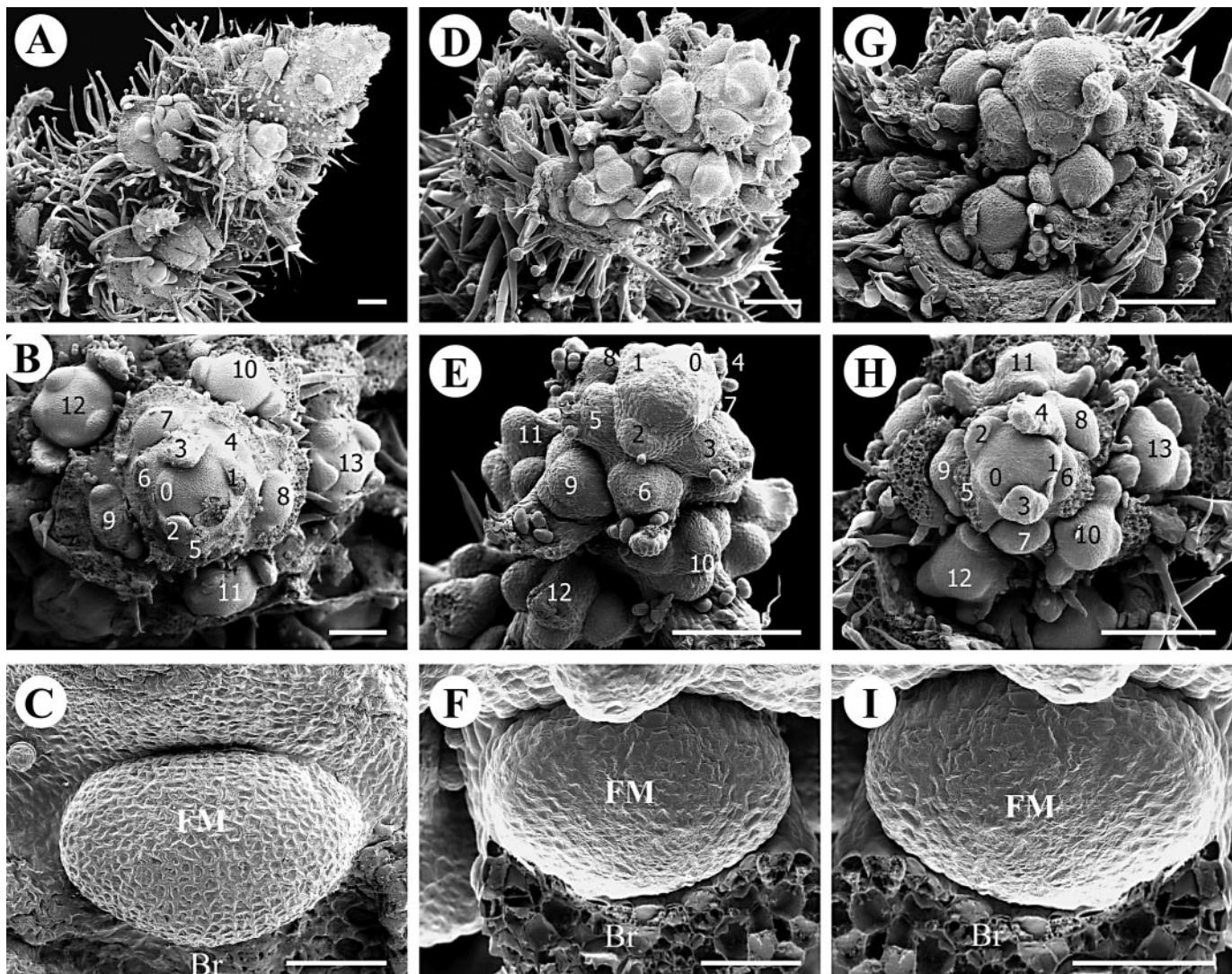


Fig. 3. The apex of three different inflorescence types and floral meristem (FM) development in *Titanotrichum*. (A, B, C) Flower formation; (D, E, F) bulbil clustering; and (G, H, I) bracteose proliferation. The floral meristems remain indistinguishable up to the loaf stage, as seen in C, F, and I. The primary floral meristems are denoted by FM, subtending bracts by Br, and successively older bracts and associated floral meristems from the shoot apex are numbered 1–13 in B, E, and H. Bar = 50 μm in C, F, I; other bars = 200 μm .

as *Antirrhinum*, these floral primordia would develop straightforwardly into flowers with whorls of floral organs arising from the meristems sequentially (e.g., Bradley et al., 1996a). However, in *Titanotrichum* the developmental fate of these meristems is labile, changing during the season. Three different fates can be observed at the apex of the inflorescence (Fig. 3): (1) flower formation (Fig. 3B), (2) bulbil formation (Fig. 3E), and (3) bracteose proliferation (Fig. 3H). Transitional states and reversals are also seen, suggesting that the meristems are uncommitted in very early development.

When flowers are to be formed, the shoot apex elongates and well-spaced floral primordia are initiated (Fig. 3A, B). The “pentagon-shaped” primordium appears at node 10–12 and indicates pentamerous organ-whorl development and hence flower induction (Fig. 3B). The terminology of floral stages used here follows that used for *Antirrhinum* (Carpenter et al., 1995). If meristems switch to bulbil production and bracteose proliferation, shoot apex organogenesis appears more com-

pressed. Bulbil primordia are distinguishable from true floral primordia shortly after the “loaf” stage (node 5–7) (Fig. 3C, F, I), as the “pentagon” stage does not form. Instead, in bulbil development, three subsidiary meristems form (node 9–10) (Fig. 4E, F: m1–m3). In bracteose proliferation, more than two bract primordia initiate (Fig. 4I–L, $\alpha + \beta$).

Floral development—When the floral meristem enters the floral development pathway, the primordium is at first laterally elongated (Fig. 4A). It then produces two opposite bracteoles in succession, which remain slightly unequal in size (Fig. 4A). The floral apex then becomes pentagonal, and all the sepals are initiated synchronously, although the abaxial two are somewhat larger (Fig. 4B–D). Later, the five petal primordia are initiated nearly synchronously, alternating with the sepals (Fig. 4M). The four functional stamen initials appear at almost the same time as the petal primordia (the single staminode arises later) (Fig. 4N–P).

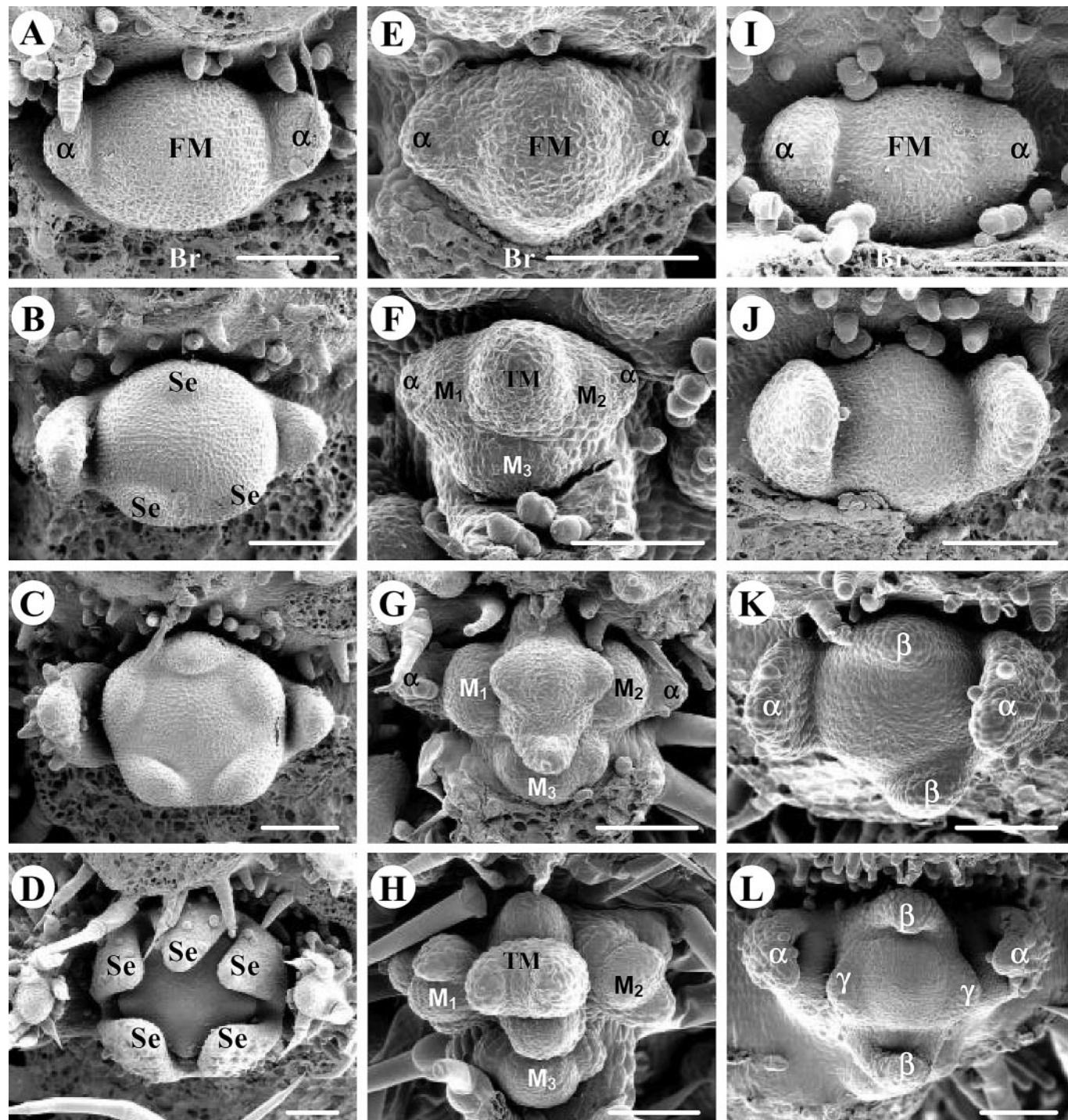


Fig. 4. Scanning electron micrographs of the developing floral meristems. (A–D) Flower formation; (E–H) bulbil cluster formation; (I–L) bracteose proliferation. Abbreviations: FM = the primary floral meristem, Se = sepals, Br = subtending bract, α , β , γ = bracteoles (first, second, and third pairs, respectively). In the production of bulbil clusters, the floral meristem has been divided into terminal and three subsidiary meristems, which are denoted by TM, M_1 , M_2 , and M_3 . See text for explanation. Bars = 100 μm . (M–X) Later stages of developing floral meristems. (M–P) Flower formation; (Q–T) bulbil proliferation; (U–X) bracteose proliferation. Abbreviations: C = petals, S = stamen, St = staminode, G = gynoecium. In bulbil cluster formation, the terminal (TM) and three subsidiary meristems (M_1 – M_3), eventually form four major primordia (P_0 – P_3) for clusters of bulbils. In bracteose proliferation, a fourth pair of bracteoles are formed (δ), in addition to α , β , and γ . Bars = 100 μm .

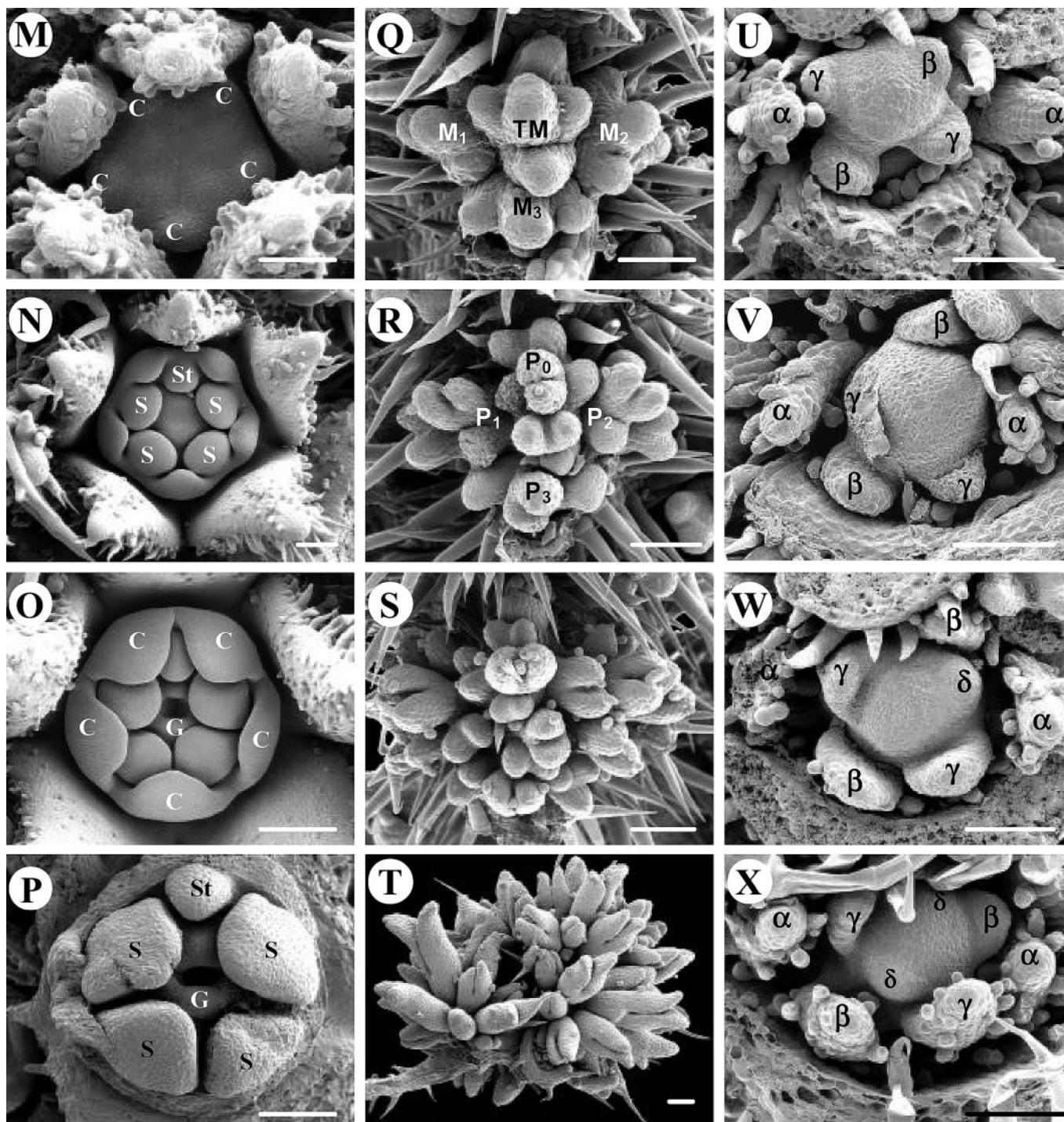


Fig. 4. Continued.

Development of bulbil clusters and the phenomenon of bracteose proliferation—The floral meristems of *T. oldhamii* can apparently convert to the production of bulbil primordia or into multibract units immediately after their two lateral bracteoles initiate (Fig. 4I, K). When primary meristems enter the bracteole-producing developmental pathway, the two bracteoles continue to enlarge, and the meristems continue to produce more bracteoles (Fig. 4L, U–X). This phenomenon we call “bracteose proliferation,” and it does not lead to the pro-

duction of any reproductive units. Bracteose proliferation appears to be an intermediate state between pathways for flower and bulbil production, as it entails production of bracts like those associated with flowers, but proliferation of units similar to that associated with bulbil production. In addition, bracteose proliferation usually occurs temporally between flower and bulbil production.

In contrast, the initiation of bulbils is more complicated. After the two bracteoles develop, three subsidiary meristems

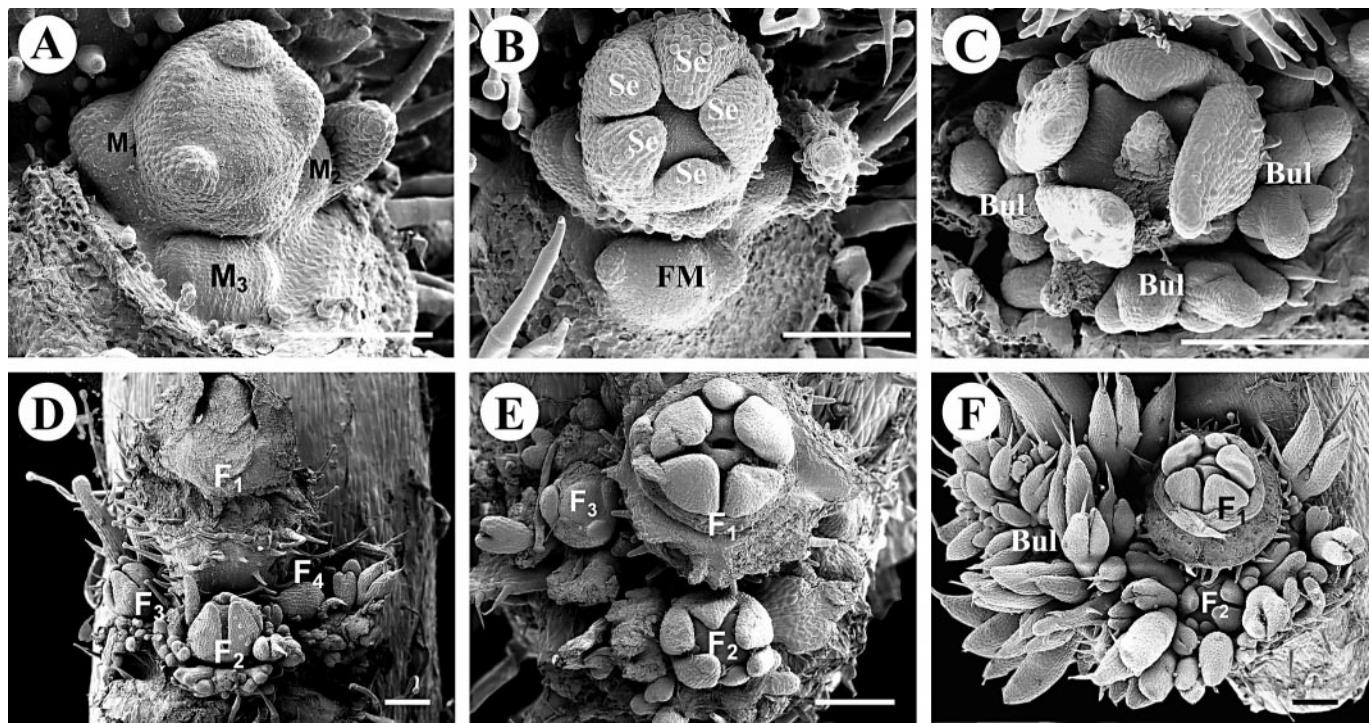


Fig. 5. (A–F) Flowers and bulbil clusters within the same floral meristem. Up to four flowers (F_1 – F_4) may initiate with bulbils (Bul) surrounding them. Bars = 200 μ m. See Fig. 4 for meaning of other abbreviations.

arise laterally to the primary meristem in the axils of the bract and bracteoles (Fig. 4F, G). Then all the meristems proliferate (two new meristems arise on the flanks of existing meristems repeatedly) to generate the numerous bulbil primordia (Fig. 4H, Q–S). Thus, each floral meristem might give rise eventually to 50–70 bulbils (Fig. 4T). Bulbils can also arise from new meristems formed in the axils of bracts and bracteoles as well as by proliferation of the main axillary meristem.

It is possible to find mixed conditions in which flowers and bulbils have both arisen from the same meristem (Fig. 5), indicating that meristems are not committed to a single pathway at initiation. One to four flowers with associated surrounding bulbils may arise from a single floral meristem (Fig. 5C–F). These multiflowered units suggest that flowers form after the development of multiple meristems characteristic of the bulbil developmental pathway, and therefore that the bulbil developmental pathway is alterable to the floral pathway, at least at an early stage of development.

The inflorescence of *Titanotrichum* therefore varies between a simple raceme and a more complicated structure with multiple axillary meristems and inflorescence branches.

Germination of seeds and bulbils—The seeds of *Titanotrichum* are minute (~0.6 mm long, ~0.15 mm wide), narrowly ovate to spindle-shaped, with the seed coat shrunken into folds around the embryo (Fig. 6A). Seeds germinate in 7–10(–14) d at 22°C under constant climatic conditions. The germination rate is very variable but is usually about 75%. With the elongation of the hypocotyl, the radicle normally emerges through the micropylar region 7–8 d after sowing (Fig. 6D). Thereafter, the hypocotyl continues to elongate, and a ring of rhizoids is formed at the base of the hypocotyl. These rhizoids probably assist in water absorption before the primary root develops and

serve to anchor the seedling (Fig. 6B, C). At ~2 wk the cotyledons are fully expanded and green. Many Old World Gesneriaceae show accrescent growth of one cotyledon (anisocotyly). In *Titanotrichum* most of the seedlings expand their cotyledons at the same rate during their growth, although some (20–40%) had slightly unequal expansion at a very early stage of germination, apparently due to an initial difference in cotyledon size. True anisocotyly shows accrescence in one cotyledon after initial cotyledon expansion, due to the formation of a basal meristem in one cotyledon. This does not appear to happen in *Titanotrichum*, which is therefore strictly isocotylous. After cotyledon expansion, the primary root elongates and the first pair of true leaves is initiated (Fig. 6E). Although the adult plant is densely covered with multicellular hairs, the seedling has a few glandular hairs only.

Compared to seeds, bulbils “germinate” very easily, taking 1 wk and, unlike seedlings, can live for 1–2 mo on wet filter paper without nutrients. The bulbil germination rate is higher than that of seeds, at ~95%.

Bulbil germination in *Titanotrichum* is somewhat different to that of seedlings. Each bulbil has two “storage” bracts and oil bodies are visible in this tissue as droplets within the cells (in cross section under the light microscope). These bracts form a V-shape at the top of a short “bract stem” (Fig. 6F). In comparison to the seed, it is relatively large (1.5 mm long, 0.5 mm wide). When a bulbil is about to germinate, it forms a ring of rhizoids to take up water (like those in true seedlings) (Fig. 6G, H). Then, on the side of the bract stem, a root primordium begins to initiate (Fig. 6H). Furthermore, a first pair of leaves (“bulbil leaves”) start to develop between the two storage bracts (Fig. 6I). The bulbil leaves continue to grow on an elongating “bulbil internode” (Fig. 6I). A second pair of

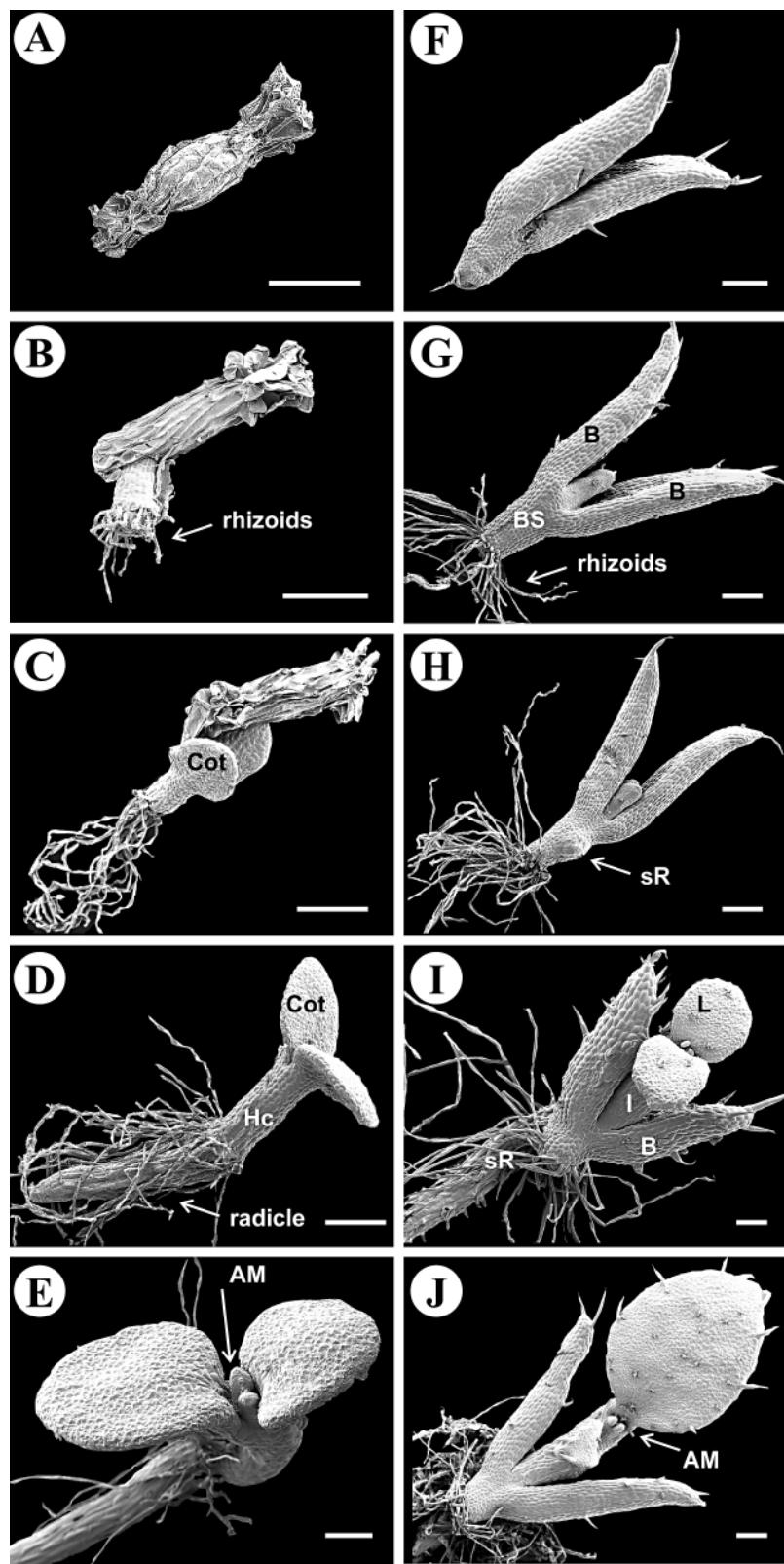


Fig. 6. The process of seed (A–E) and bulbil (F–J) germination. Abbreviations: Cot = cotyledons, Hc = hypocotyl, B = bracts, BS = bract stem, sR = bulbil root, L = bulbil leaves, I = bulbil internode, and AM = apical meristem. Bars = 200 μm .

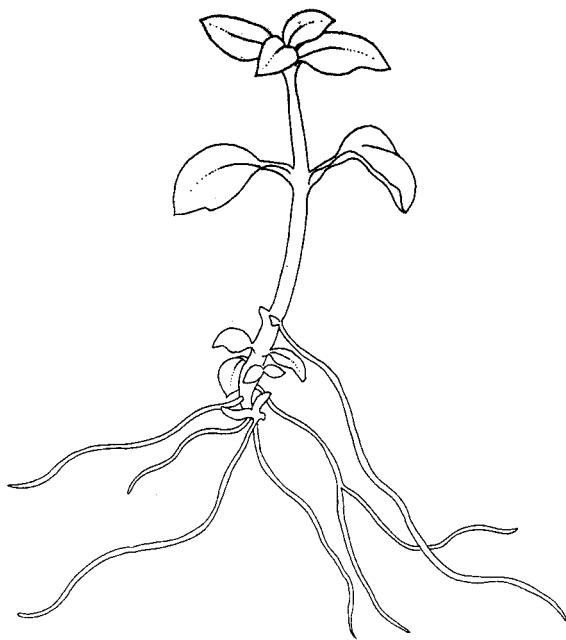


Fig. 7. A bulbil-germinated "seedling." The remains of the V-shaped bulbil can be seen at the base.

leaves arises from the apical meristem between the bulbil leaves 1 wk after the start of germination (Figs. 6J, 7).

Environmental control—In the field, bulbil production of *Titanotrichum* begins at the end of summer, apparently when daylength begins to shorten. Around the end of August, daylength in its native habitat declines slowly to <12 h (eventually to 10 h on the shortest day of the year). The mean daily temperature falls too, typically from 30°C to 22°C, but seldom drops below 20°C. To investigate the control of bulbil production further we made observations on 4–5 individuals of each of six clones (from five populations) grown under controlled conditions. In every case when offspring from the same genotype were placed into different daylength/temperature growth cabinets, plants in long daylength conditions and low temperature (16 h daylight and constant temperature at 18°C) had slowed floral development but no inhibition of the initiation and development of bulbil inflorescences. Individuals in artificial long day conditions but moderate temperature (16 h daylight and 23°C) reduced production of bulbiliferous inflorescences, and flowering inflorescences predominated. In short days, flowers are not produced, but bulbils are produced vigorously at both temperatures (8 h daylight).

DISCUSSION

Plasticity of meristem behavior—*Titanotrichum* has inflorescence meristems that can revert to vegetative growth (bulbil formation and bracteose proliferation) (Fig. 8). Moreover, many individuals can produce bulbil-only inflorescences while other inflorescences are still flowering. Occasionally, individuals may have their inflorescences transformed from flower production (with flowers at the base) to the production of bulbil primordia or bracteose proliferation (sometimes the multibracteole units may produce enlarged bracts or even leaves) and then revert back to flowering again at the top. This variation suggests that after inflorescence induction, the inflores-

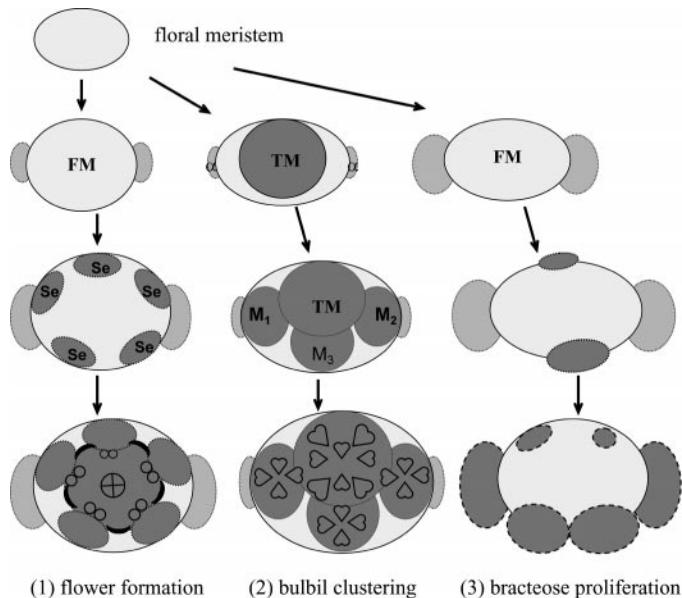


Fig. 8. Meristem fate during development in three different pathways: flower formation, bulbil proliferation, and bracteose proliferation. Pale shading indicates bracts, darker shading meristems. See Fig. 4 for meaning of abbreviations.

cence meristem of *Titanotrichum* is constantly regulated by the interaction of genetic and environmental signals.

The floral and bulbil meristems are indistinguishable up to the end of the loaf stage (Fig. 3C, F, I). Apparently, the meristems are uncommitted at this early stage and are developmentally plastic when formed. There seems to be a directional phase change in the fate of primordia within the inflorescence: flowers form at the beginning of the flowering season, then bulbil formation replaces flowers at the top of the inflorescence. Bulbils are also produced from the axillary meristems in the bracts and bracteoles of existing flowers. The existence of multiflower units, formed after the multiple meristems have developed at the start of the bulbil pathway, suggests that the meristems are not irrevocably committed at this stage and can revert back to flower formation. Occasionally, bracteose proliferation occurs, in which multiple bracteoles form in a whorl surrounding the undifferentiated floral meristem.

The meristem plasticity of *Titanotrichum* is perhaps unsurprising because the Gesneriaceae as a whole displays unusually variable meristem behavior. Some genera of the Gesneriaceae, such as *Streptocarpus* (Jong and Burtt, 1975; Möller and Cronk, 2001), vary enormously in basic morphology. Three major growth forms occur in *Streptocarpus*: caulescent, unifoliate, and rosulate (Jong and Burtt, 1975). Unifoliates have no shoot apical meristem. Instead, they have a single cotyledon with a basal meristem and a separate meristem in the midrib. The variable behavior of the vegetative meristem in *Streptocarpus* is uncoupled from that of the reproductive meristems, however. Reproductive meristems remain unchanged, producing normal pair-flower cymes like other members of the Gesneriaceae.

Developmental switches and meristem transitions—Developmental and genetic studies on meristem behavior in mutants of *Antirrhinum* and *Arabidopsis* provide cases analogous to that in *Titanotrichum*. For instance, the *squamosa* (*squa*) mu-

tant of *Antirrhinum majus* L. is characterized by excessive formation of bracts and the production of fewer and deformed flowers (Huijser et al., 1992). Similarly, the *floricaula* (*flo*) mutant of *Antirrhinum*, homologous to the *leafy* (*lfy*) mutant of *Arabidopsis*, produces indeterminate shoots bearing further bracts (instead of flowers) in the axils of bracts (Coen et al., 1990). These phenotypes combined (as in the *flo/squa* double mutant) are similar to the bracteose proliferation form in *Titanotrichum*. The development of the floral meristem in the *squa* or *flo* mutant is similar to the wild type until the loaf stage, but the meristem then fails to form sepals in a whorl. Instead, bracteole primordia form at the end of the loaf structure. This meristem may give rise to an indeterminate inflorescence with a spiral array of bracteoles (*flo*) or remain with two lateral bracteoles plus two ventral primordia without floral parts (*squa*).

Because these phenotypes are artificial mutations, they are not developmentally plastic as in *Titanotrichum*. Nevertheless, the similarity of developmental stages in both *flo* and *squa* with the bracteose proliferation form of *Titanotrichum* is striking (Figs. 4X, 8). Thus Gesner-*FLO* and Gesner-*SQUA* may be candidate genes for the regulation of the bracteose proliferation phenotype in *Titanotrichum*.

The cluster of bulbil primordia produced at one floral meristem can be likened to a compressed inflorescence side branch (Fig. 4G). Again the Gesner-*FLO* gene may play a role in this because *FLO* promotes transitions between floral and vegetative (branching) phases. Moreover, the *TERMINAL FLOWER-1* (*TFL-1*) gene of *Arabidopsis* (Araki, 2001) and the homologous *CENTRORADIALIS* (*CEN*) gene of *Antirrhinum* are known to influence whether the inflorescence is determinate or indeterminate (Bradley et al., 1996b). Overexpression of *TFL-1* in *Arabidopsis* results in a prolonged vegetative phase and a highly branched inflorescence (Ratcliffe et al., 1998; Schmitz and Theres, 1999). Similarly, work on meristem reversion of *Impatiens* suggests that *FIMBRIATA* (*FIM*) affects the formation of the whorled phyllotaxy and defines the boundaries of different organ-identity genes (Pouteau et al., 1998a). Environmental changes can cause the *Impatiens* *bal-samina* L. flower axis to revert back to a vegetative meristem, resulting in deformed flowers and leaves (bracts) (Pouteau et al., 1997, 1998b). Floral meristems in *Impatiens* can therefore also adopt different fates.

Bulbil formation is scattered widely in angiosperms. Many species, such as *Saxifraga cernua* (Saxifragaceae), *Ranunculus ficaria* (Ranunculaceae), and *Remusatia vivipara* Schott (Araceae) share with *Titanotrichum* the ability to initiate bulbils in place of the floral meristem (Kerner, 1904). *Mimulus gemmiparus* too, although it produces its propagules from lateral meristems (the distal axillary bud adjacent to the floral bud), has an essentially similar pattern of development (Moody et al., 1999). Thus there may be an analogous developmental switch common to bulbil formation in diverse species. *Titanotrichum* is unusual in that this developmental switch incorporates successive meristem divisions to produce numerous bulbils from one primordium. Nothing comparable to this is found elsewhere in Gesneriaceae.

Similarity between bulbils and seedlings—With the obvious exception of the two prominent bracts, the *Titanotrichum* V-shaped bulbil possesses a number of similarities to the seeds. On “germination,” a pair of bulbil leaves arises on an elongated bulbil-internode (Fig. 6I). Root initiation in the bul-

bil is secondary (the root primordium grows endogenously from the side of the bract stem). On the other hand, the seed germinates with a true radicle (primary root), which is persistent in *Titanotrichum*, although the development of a secondary root seedling, after primary root abortion, is common in many other Old World Gesneriaceae species (Fig. 6D and E). There is no “anisocotylly” in bulbil germination, the bract and leaf pairs produced on germination being equal. Wang et al. (2002) recently observed seedlings from two individuals of *T. oldhamii* and concluded that it is anisocotylous. Our observations have not revealed any differential growth of the cotyledons after initial expansion, resulting from the action of a basal meristem (anisocotylly). The initial expansion may however be slightly unequal. To clarify this, it would be useful to examine whether there is an unequal cell division rate in the two cotyledons, as demonstrated by Tsukaya (1997) in *Monophyllaea*.

Moody et al. (1999), following Troll, defined the vegetative propagules of *Mimulus gemmiparus* as brood bulbils (with storage in the leaf component), distinct from brood tubers (storage in the stem component). The storage bracts of *Titanotrichum* are part of the wide range in form of the storage organs of vegetative propagules. For instance, in Dioscoreaceae (Passam et al., 1982) and in *Globba* (Zingiberaceae), the vegetative propagules are tuber-like, while in *Allium* (Alliaceae), globular propagules form at the base of umbel pedicels. The propagules of *Saxifraga* (Saxifragaceae) have enclosing bracts and replace flowers within the inflorescence. *Mimulus gemmiparus* (Scrophulariaceae) closely parallels *Titanotrichum* by possessing V-shaped storage bracts (Moody et al., 1999). In *Titanotrichum* however, the whole floral meristem has been replaced by a cluster of bulbils, allowing *Titanotrichum* to produce large numbers of bulbils, whereas in *Mimulus* (Moody et al., 1999), only one propagule arises (from the proximal axillary buds).

Ecological significance of bulbil production—Bulbil production is the common state of all natural populations of *Titanotrichum* when autumn approaches (at the beginning of September). Because *Titanotrichum* usually grows near water, these tiny bulbils disperse by flotation very easily. When attempting to trace the origin of young clonal populations along ditches or tributaries, it is always possible to locate a putative progenitor colony or plant upstream. Bulbils may also be more easily carried by animals (including humans) than seeds. The pointed storage bracts, which have long trichomes, readily catch on human clothing and probably on animal fur. Plants that regenerate from bulbils grow vigorously and establish quickly. They can produce rhizomes in a shorter period than seedlings, which is important for surviving drought or the death of aboveground growth. Although flowers are produced freely in almost all natural populations, seeds are hardly ever set. Propagation by bulbils is thus the major means of mass reproduction and dispersal for *Titanotrichum* in the wild, especially since seeds are rarely set, perhaps because of a lack of effective pollinators (C.-N. Wang, personal observation).

Bulbils are unusual in tropical or subtropical plants (with certain exceptions such as *Remusatia vivipara*). *Titanotrichum* is therefore interesting in being a subtropical plant that uses bulbil propagation as its main reproductive strategy. *Titanotrichum* often grows in dense shade in which inflorescence growth and flower production is reduced. Individuals under dense shade tend to produce more bulbils relative to flowers

compared to individuals in full light. Furthermore, field observations suggest that seedlings rarely establish in deep shade, and most regeneration is by the more robust bulbil-produced juvenile plants and by rhizomatous spread.

It is notable that bulbil formation in glasshouse conditions in Edinburgh is much more pronounced than that in the wild. Almost every leaf axil initiates a bulbil inflorescence resulting in massive bulbil production. We attribute this to low glasshouse temperatures ($\sim 18^{\circ}\text{C}$ maximum) and to the rapid decline of daylength after mid-autumn that occurs in Edinburgh. From observations in the wild and from examination of herbarium specimens collected in autumn and winter in Taiwan, the situation in the wild is less marked, and bulbiliferous inflorescences do not so dramatically replace normal flowers and vegetative shoots.

Significance of bulbil production for conservation—Seed set is very low in natural populations of *Titanotrichum* in Taiwan, adjacent regions of China, and the Ryukyu Islands of Japan, which suggests that bulbil production is the major reproductive strategy for nearly all populations despite regular flower production. In the open, large bees and butterflies sometimes visit the flowers. However, in deep shade, insects rarely visit *Titanotrichum* flowers, even though other flowering plants nearby, such as *Begonia* spp., are regularly visited by pollinating insects. Although sexual reproduction appears to be infrequent, occasional seed set and gene flow between populations may be important in maintaining population viability. Surveys of the genetic variation within and between populations are needed in order to determine the extent of clonality and ascertain whether the genetic variation is lower in marginal or threatened populations.

LITERATURE CITED

- ARAKI, T. 2001. Transition from vegetative to reproductive phase. *Current Opinion in Plant Biology* 4: 63–68.
- ARIZAGA, S., AND E. EZCURRA. 1995. Insurance against reproductive failure in a semelparous plant: bulbil formation in *Agave macroacantha* flowering stalks. *Oecologia* 101: 329–334.
- BRADLEY, D., R. CARPENTER, L. COPSEY, C. VINCENT, S. ROTHSTEIN, AND E. COEN. 1996a. Control of inflorescence architecture in *Antirrhinum*. *Nature* 379: 791–797.
- BRADLEY, D., C. VINCENT, R. CARPENTER, AND E. COEN. 1996b. Pathways for inflorescence and floral induction in *Antirrhinum*. *Development* 122: 1535–1544.
- BRIGGS, D., AND S. M. WALTERS. 1997. Plant variation and evolution, 3rd ed. Cambridge University Press, Cambridge, UK.
- BURTT, B. L. 1970. Studies in the Gesneriaceae of the Old World XXXI: some aspects of functional evolution. *Notes of the Royal Botanic Garden Edinburgh* 30: 1–10.
- CARPENTER, R., L. COPSEY, C. VINCENT, S. DOYLE, R. MAGRATH, AND E. COEN. 1995. Control of flower development and phyllotaxy by meristem identity genes in *Antirrhinum*. *Plant Cell* 7: 2001–2011.
- COEN, E. S., J. M. ROMERO, S. DOYLE, R. ELLIOTT, G. MURPHY, AND R. CARPENTER. 1990. *floricaula*: A homeotic gene required for flower development in *Antirrhinum majus*. *Cell* 63: 1311–1322.
- DIGGLE, P. K. 1997. Extreme preformation in alpine *Polygonum viviparum*: an architectural and developmental analysis. *American Journal of Botany* 84: 154–169.
- ENGELL, K. 1973. A preliminary morphological, cytological, and embryological investigation in *Polygonum viviparum*. *Botanisk Tidsskrift* 67: 305–316.
- HAYATA, B. 1908. Flora montana Formosae. *Journal of the College of Science in Imperial University Tokyo* 25: 174.
- HAYATA, B. 1911. Material for a flora of Formosa. *Journal of the College of Science in Imperial University Tokyo* 30: 211–212.
- HAYATA, B. 1912. *Icones Plantarum Formosanarum*. Government of Formosa, Taipei, Taiwan.
- HEMSLEY, W. B. 1890. An enumeration of all the plants known from China proper, Formosa, Hainan, Corea, the Luchu Archipelago, and the island of Hongkong, together with their distribution and synonymy. *Journal of the Linnean Society* 26: 121–236.
- HEMSLEY, W. B. 1895. Descriptions of some new plants from eastern Asia, chiefly from the island of Formosa, presented by Dr. Augustine Henry, F.L.S., to the Herbarium, Royal Gardens, Kew. *Annals of Botany* 33: 143–160.
- HEMSLEY, W. B. 1909. Miscellaneous information. *Royal Botanic Gardens Kew Bulletin of Miscellaneous Information* 9: 357–361.
- HENRY, A. 1898. A list of plants from Formosa. *Transactions of the Asiatic Society of Japan* 24: 68.
- HUIJSER, P., J. KLEIN, W.-E. LÖNNIG, H. MEIJER, H. SAEDLER, AND H. SOMMER. 1992. Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *squamosa* in *Antirrhinum majus*. *EMBO Journal* 11: 1239–1249.
- IMAICHI, R., S. NAGUMO, AND M. KATO. 2000. Ontogenetic anatomy of *Streptocarpus grandis* (Gesneriaceae) with implications for evolution of monophyllly. *Annals of Botany* 86: 37–46.
- JONG, K., AND B. L. BURTT. 1975. The evolution of morphological novelty exemplified in the growth patterns of some Gesneriaceae. *New Phytologist* 75: 297–311.
- KERNER, A. 1904. The natural history of plants, their forms, growth, reproduction and distribution. Gresham, London, UK.
- MÖLLER, M., AND Q. C. B. CRONK. 2001. Evolution of morphological novelty: a phylogenetic analysis of growth patterns in *Streptocarpus* (Gesneriaceae). *Evolution* 55: 918–929.
- MOODY, A., P. K. DIGGLE, AND D. A. STEINGRAEBER. 1999. Developmental analysis of the evolutionary origin of vegetative propagules in *Mimulus gemmiparus* (Scrophulariaceae). *American Journal of Botany* 86: 1512–1522.
- PAN, K.-Y., Z.-Y. LI, AND Y.-Z. WANG. 2002. Floral organogenesis of *Titanotrichum oldhamii* (Gesneriaceae). *Acta Botanica Sinica* 44: 895–902.
- PASSAM, H., L. D. WICKHAM, AND L. A. WILSON. 1982. Comparative observations on the polarity of sprouting of bulbils of *Dioscorea bulbifera* L. and *Dioscorea alata* L. *Annals of Botany* 49: 359–366.
- POUTEAU, S., D. NICHOLLS, F. TOOKE, E. COEN, AND N. BATTEY. 1997. The induction and maintenance of flowering in *Impatiens*. *Development* 124: 3343–3351.
- POUTEAU, S., D. NICHOLLS, F. TOOKE, E. COEN, AND N. BATTEY. 1998a. Transcription pattern of a FIM homologue in *Impatiens* during floral development and reversion. *Plant Journal* 14: 235–246.
- POUTEAU, S., F. TOOKE, AND N. BATTEY. 1998b. Quantitative control of inflorescence formation in *Impatiens balsamina*. *Plant Physiology* 118: 1191–1201.
- RATCLIFFE, O. J., I. AMAYA, C. A. VINCENT, S. ROTHSTEIN, R. CARPENTER, E. S. COEN, AND D. J. BRADLEY. 1998. A common mechanism controls the life cycle and architecture of plants. *Development* 125: 1609–1615.
- SCHMITZ, G., AND K. THERES. 1999. Genetic control of branching in *Arabidopsis* and tomato. *Current Opinion in Plant Biology* 2: 51–55.
- SEALY, J. R. 1949. *Titanotrichum oldhamii*. *Botanical Magazine* 166: pl. 78 [1–6].
- SOLEREDER, H. 1909. Über die Gattung *Rehmannia*. *Berichte der Deutschen Botanischen Gesellschaft* 27: 390–404.
- STAPF, O. 1911. Figures, with descriptive characters and remarks of new and rare plants. *Hooker's Icones Plantarum* 30: Tabula 2936.
- TROLL, W. 1964. Die Infloreszenzen: Typologie und Stellung im Aufbau des Vegetationskörpers. Gustav Fischer, Jena, Germany.
- TSUKAYA, H. 1997. Determination of the unequal fate of cotyledons of a one-leaf plant, *Monophyllaea*. *Development* 124: 1275–1280.
- VINCENT, C. A., R. CARPENTER, AND E. S. COEN. 1995. Cell lineage patterns and homeotic gene activity during *Antirrhinum* flower development. *Current Biology* 5: 1449–1458.
- WALKER, E. H. 1976. Flora of Okinawa and the southern Ryukyu islands. Smithsonian Institution Press, Washington, D.C., USA.
- WANG, W.-T., K.-Y. PAN, Z.-Y. LI, A. L. WEITZMAN, AND L. E. SKOG. 1998. Gesneriaceae. In Z. Y. Wu and P. H. Raven [eds.], *Flora of China*, vol. 18, 244–401. Science Press & Missouri Botanical Garden Press, St. Louis, Missouri, USA.
- WANG, Y.-Z., Z.-Y. LI, K. Y. PAN, AND X.-H. ZOU. 2002. Pattern and significance of seedling development in *Titanotrichum oldhamii* (Gesneriaceae). *Acta Botanica Sinica* 44: 903–907.

- WEBER, A. 1973. Die Struktur der paarblutigen Partialfloreszenzen der Gesneriaceen und bestimmter Scrophulariaceen. *Beitrage Zur Biologie der Pflanzen* 49: 429–460.
- WEBER, A. 1978. Transitions from pair-flowered to normal cyme in Gesneriaceae. *Notes Royal Botanic Garden Edinburgh* 36: 355–368.
- WEBER, A. 1982. Evolution and radiation of the pair-flowered cyme in Gesneriaceae. *Australian Systematic Botanical Society Newsletter* 30: 23–41.
- WEBER, A. 1995. Developmental aspects of the pair-flowered cyme of Gesneriaceae. *Gesneriana* 1: 18–28.
- WEBER, W. A. 1972. *Mimulus gemmiparus* sp. nov. from Colorado. *Madrone* 21: 423–425.
- YOUNGNER, V. B. 1960. Environmental control of initiation of the inflorescence, reproductive structures and proliferations in *Poa bulbosa*. *American Journal of Botany* 47: 753–757.