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Reversion of floral development under adverse ecological conditions in *Whytockia bijieensis* (Gesneriaceae)

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Abstract. The reversion of floral development in *Whytockia bijieensis* Y.Z.Wang et Z.Y.Li occurs after the normal initiation of mid-adaxial sepals, which implies that the floral organs are not determined at the initiation of the floral primordium, but progressively determined during its development. Both low humidity (less than 75%) and low night temperature (12–14°C) contribute to the reversion of floral development. However, the low humidity or low night temperature alone do not greatly affect the normal flowering process. The combination of these two ecological factors seems to considerably disturb the normal flowering process, greatly strengthening the reversion process of flowering. High humidity and moderate temperature or at least one of the two are necessary for the normal flowering process of *W. bijieensis*. It is likely that low humidity and low night temperature trigger the reversal to vegetative growth during floral development. The change of a flower into a bract-like organ in the flower pairs indicates that a transition from pair-flowered to normal cymes is possible.

Introduction

According to the review by Bettey and Lyndon (1990), reversion of flowering in many plants is under environmental control, and reflects the potential that many plants have for their meristems to grow vegetatively after a period of flowering. It is brought about by different ecological factors in different species, and is very likely to occur when plant growth has been disturbed by very unusual environmental or out-of-season conditions. *Whytockia*, part of a relict group in the Epithemateae (Gesneriaceae) (Weber 1982), mainly survives in humid habitats under dense forests in the subtropical mountains in South-west China. According to my observations in the natural habitat, local populations of *W. bijieensis* have been found to grow by streams in dense forests at an altitude of about 1600 m, and the anthesis of this species is during the rainy season with high humidity and moderate temperature. The environmental conditions of the dry season in the natural habitat are remarkably variable in temperature and humidity. Whether the high humidity and moderate temperature are necessary for the flowering of *W. bijieensis* is not known, as the interpretation and analyses of insufficient experimental data are difficult.

In addition, the relationship between pair-flowered and normal cymes is important in the phylogeny of the family Gesneriaceae (Weber 1978). The pair-flowered cyme exhibits an unorthodox structure in that each cyme unit ends with a flower pair: a terminal and a frontal flower rather than only a terminal flower in the normal cyme. Weber (1978) suggested that the normal cyme was derived from the pair-flowered cyme through a process from slight inhibition to complete suppression of the frontal flowers. *Whytockia* is characterised by ebracteate and simple pair-flowered cymes. A change from flower to bract has been observed in a flower pair of *W. bijieensis* during the late period of anthesis in the natural habitat (Wang and Li 1997). However, it is unknown whether it indicates a cue of a transition from pair-flowered to normal cymes or an occasional event, and under which environmental factor or factors it occurs.

The aim of this study is, first, to try to reveal the ecological factors controlling the flowering process in *W. bijieensis*, and, second, to provide insights into the ecological background of the change between vegetative and floral organs in Gesneriaceae.

Materials and methods

Plant materials

At the end of the growing season in October, the perennial plants of *W. bijieensis* Y.Z.Wang et Z.Y.Li were transplanted from the natural habitat into the pots in the Kunming Botanical Garden. The experiment was carried out in October the following year, during the late period of anthesis.

Environmental and experimental conditions

Under natural conditions, anthesis starts in June and ends in October. During this period, the day/night temperatures were 26–30 and 20–25°C, respectively, with relative humidity greater than 95%.

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At the Kunming Botanical Garden, the climate was similar to that of the natural location except that the dry season came earlier. During the experimental period, the day temperature in the garden was 25–28°C and the night temperature and relative humidity were 12–14°C and less than 75%, respectively.

The plants in the cultivation experiment were divided into four groups and each group contained six individuals. The first group was cultivated in a shaded greenhouse which was kept in a similar condition to that of the natural habitat (day/night temperature 26–28/22–24°C and relative humidity above 95%). The second group was treated similarly except for uncontrolled temperature (see above). The temperature in the third group was controlled to a day/night interval of 26–28/22–24°C, while the relative humidity was maintained at less then 75%. The fourth group was cultivated outdoors with the night temperature of 12–14°C and a relative humidity less than 75%, but shaded similarly as the other three groups.

Flower materials for scanning electron microscopy (SEM)

Samples representing floral development under natural conditions were collected from plants at the natural location (Bijie, Guizhou Province, China), and samples of plants cultivated under adverse conditions (Groups 2–4) at the Kunming Botanical Garden. Buds at different developmental stages were fixed in formalin : acetic acid : alcohol (FAA, 5 : 6 : 89), and examined in 70% ethanol under a dissecting microscope. Materials were further microdissected in 95% ethanol and then dehydrated in three changes of 100% ethanol, 100% ethanol + amylacetate (1 : 1) and amylacetate, critical point dried with a Hitachi HCP-2 Critical Point Dryer and then Au/Pd Sputter coated with a SPI-MODULE Sputter Coater. The prepared samples were examined with a Hitachi S-800 Scanning Electron Microscope and recorded in Shanghai Panchromatic film.

Results

Normal development of inflorescences and flowers

The aerial part of *W. bijieensis* branches in a sympodial way in which each new shoot meristem produces a pair of unequal opposite leaves (Fig. 1). After a period of vegetative growth, inflorescence primordia successively arise in the axil of large leaves (Fig. 1). At first, the inflorescence primordium develops into a single flower without subtending bracts (Fig. 2). Then, a pair of flowers, which is the first flower pair of the inflorescence, is initiated at the base of the first flower (Fig. 2). In the following process, each flower pair successively and alternatively emerges at the base of the right or the left flower of the previous flower pair (Fig. 3).

A floral meristem sequentially produces four concentric whorls of floral organs: five sepals, five corolla lobes, four stamens plus one staminode and two united carpels. When the initiation of five sepals begins, the floral primordium gradually transforms into an adaxially depressed conical shape, and soon the mid-adaxial sepal arises (Fig. 2). Two adaxial-lateral sepals then appear simultaneously on either side of the mid-adaxial sepal (Figs 2, 3). When the mid-adaxial sepal nearly overarches the center of the floral apex, two abaxial-lateral sepals are initiated (Figs 2, 3). Not only the initiation but also the development of the five sepals is unidirectional from the adaxial to abaxial side (Figs 2, 3).

Aberration and reversion in the development of flowers

Aberration in development

In several cases, aberration took place during the initiation of the first flower of the inflorescence. Figure 4 shows that the five sepals of the first flower had stopped growing at an early stage. Some aberrant flowers occurred after the mid-adaxial sepal of the first flower had become visible, in which the following primordial initiation was completely repressed (Fig. 5). In some other cases, aberration occurred during the initiation and early development of the first flower pair. Sometimes, both flowers in the pair became aberrant at initiation, or one

Figs 1–12. Normal development, aberration and reversion in the development of inflorescence and flower in *Whytockia bijieensis*. **Figs 1–3.** Normal development of inflorescence and flower in *W. bijieensis*. **Fig. 1.** Polar view, showing inflorescence primordium (I) and top of vegetative shoot (SH). Scale bar: 135 μm. **Fig. 2.** Early development of inflorescence containing the first flower (FO) with five sepals unidirectionally overlapping (MS mid-adaxial sepal, DS adaxial-lateral sepal and BS abaxial-lateral sepal), and the first flower pair with adaxially depressed conical shape of the right flower primordium (SR) and successive appearance of five sepals from the adaxial to abaxial side in the left flower (SL). Scale bar: 88 μm. **Fig. 3.** The second primary flower pair primordia (TL, left flower of the second flower pair; TR, right flower of the second flower pair; and F, floral primordium), and unidirectional development of sepals in the first flower pair (SR, SL). Scale bar: 135 μm. **Figs 4–12.** Aberration and reversion in the development of inflorescence and flower in *W. bijieensis*. **Fig. 4.** Oblique view of five aberrant sepals of the first flower, and two leaf primordia of the vegetative shoot (SH). Scale bar: 93 μm. **Fig. 5.** An aberrant first flower takes place at an early stage of floral development. Scale bar: 56 μm. **Fig. 6.** Lateral view, a single aberrant flower depressed after early development of the mid-adaxial sepal (MS) at the second node of the inflorescence. Scale bar: 75 μm. **Fig. 7.** Oblique polar view of the first flower pair, one aberrant during early development, the other with a normally developed calyx (five sepals have been removed) and the primordia of corolla and androecium. Scale bar: 142 μm. **Fig. 8.** Oblique view of a reversed flower at left accompanied by an aberrant flower at right, with the normally initiated mid-adaxial sepal (MS) and the first leaf-like primordium (A) emerging at right side of the floral apex, below and at an approximately 90° angle to the mid-adaxial sepal (MS). Scale bar: 250 μm. **Fig. 9.** Magnification of the reversed flower in Fig. 8, showing the first leaf-like primordium (A) followed by the second leaf-like primordium (arrow head) subopposite to the mid-adaxial sepal. Scale bar: 84 μm. **Fig. 10.** Oblique view of a single reversed flower, showing the enlarged second leaf-like primordium (arrow head) and the initiation of a vegetative shoot (SH), comprising two opposite leaf primoridia on the floral apex. Scale bar: 84 μm. **Fig. 11.** Oblique view of a single reversed flower during the early development of the mid-adaxial sepal, with initiation of the first (A) and the second (arrow head) leaf-like primordium. Scale bar: 91 μm. **Fig. 12.** Different development of the first flower pair on an inflorescence axis, showing a bract-like organ (B) with uniseriate–pluricellular trichomes on its surfaces and margins below the left aberrant flower. Scale bar: 265 μm.

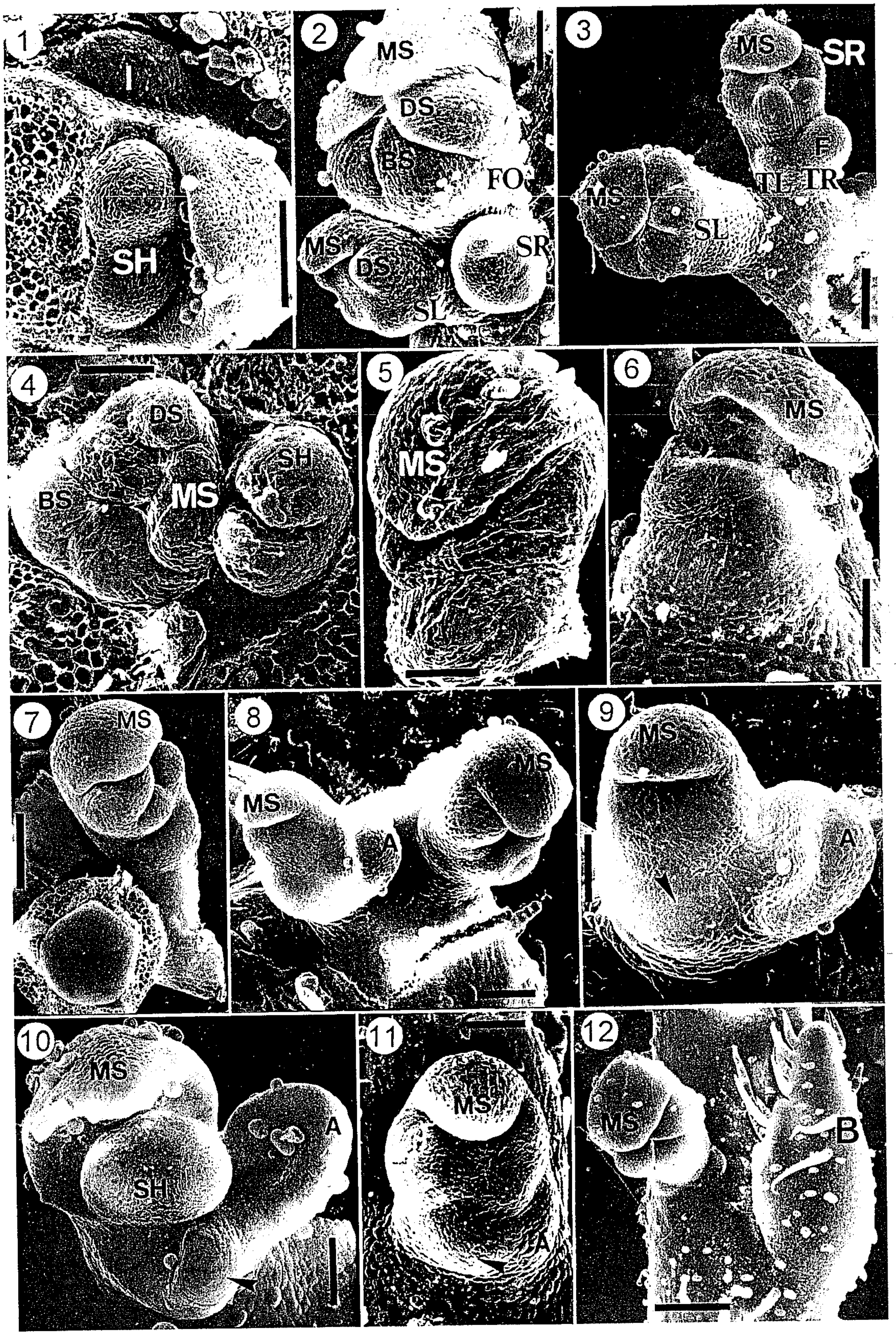
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Table 1. The number and percentage of aberrant and reversed flowers in the four experimental groups

Flowers	Group 1		Group 2		Group 3		Group 4	
	Number	%	Number	%	Number	%	Number	%
Examined	36	100	27	100	30	100	24	100
Aberrant	2	5.6	3	11.1	2	6.7	8	33.3
Reversed	0	0.0	1	3.7	3	10.0	6	25.0

flower of the pair became aberrant in the early sepal development, while the other developed normally or experienced a reversion of flowering (Figs 7, 8). As shown in Fig. 7, one flower of a pair had become aberrant during the early sepal development, while the other had a normally developed calyx and started initiation of corolla and androecium. In general, each node of the inflorescence axis bore a flower pair except for the first one. In some cases, however, only a single flower was initiated at the second node and became repressed after early development of the mid-adaxial sepal (Fig. 6).

Reversion of development

In most flowers that were reversed, the first sepal, which was the mid-adaxial sepal, arose and developed normally (Figs 8–11). However, instead of being followed by two adaxial-lateral sepals, a leaf-like primordium appeared at the righthand side of the floral apex, below and at an approximately 90° angle to the first sepal (Figs 8, 9). Next, a second leaf-like primordium arose at the lower (abaxial) side of the floral apex, subopposite to the first sepal (Figs 9, 10). Following the two leaf-like organs, a pair of leaf primordia emerged in the center of the floral apex similar to a primary vegetative shoot (Fig. 10). Sometimes, the two leaf-like primordia were initiated simultaneously after the initiation of the mid-adaxial sepal, while the two adaxial-lateral sepals were repressed (Fig. 11). Reversions of flowering always occurred during the development of the first flower pair on the inflorescence axis. In general, the reversion was accompanied by an aberrant flower (Fig. 8), but sometimes there was only a single reversed flower (Figs 10, 11).

In one extreme case, treated in the fourth group, a floral primordium completely changed into a single bract-like organ. This bract-like organ closely stuck to the inflorescence axis, which resembled a cicada on a trunk, being accompanied with a somewhat aberrant flower (Fig. 12). The aberrant flower and bract-like reversed flower were not borne at the same level on the inflorescence axis. Uniseriate–pluricellular trichoms developed on the surface and the margin of the reversed bract-like organ. At the top of this inflorescence axis, the first flower had developed normal floral organs (not shown).

Comparison of examined flowers among the four experimental groups

Among the four experimental groups, 117 flowers were examined. The number and percentage of aberrant and reversed flowers are presented in Table 1. No reversed flowers were observed in plants grown under controlled conditions similar to those of the natural habitat, whereas plants grown at low night temperature or low humidity had one and three reversed flowers, respectively. A combination of both low night temperature and low humidity resulted in six reversed flowers. The appearance of aberrant flowers showed a similar trend. Two aberrant flowers were observed in plants grown under controlled conditions similar to those of the natural habitat, while a combination of both low night temperature and low humidity led to eight aberrant flowers.

Discussion

Traditionally, flowering has been regarded as a qualitative process which, once started, normally goes to completion (Bettey and Lyndon 1990). However, in *Pharbitis*, the potential floral organs have been experimentally caused to switch to leaf development during several plastochrons after they have been initiated (King 1983). An anomalous gynoecium contains a terminal leafy shoot arising from the placenta of the ovary in *Anagallis* (Bettey and Lyndon 1990). In *W. bijieensis*, reversion occurred after normal initiation of the mid-adaxial sepal. Passing through two intermediate structures, a typical vegetative shoot (a pair of leaf primordia) arose in the center of the floral apex. The intermediate structures can be considered as a 'chaotic' state during the transition from the floral to the vegetative state. Apparently, in *Whytockia* the floral organs are not completely determined at the initiation of the floral primordium, but become progressively determined during their development.

Reversion of flowers and inflorescences occurs in response to unusual environmental conditions which are opposite to those inducing flowering (Bettey and Lyndon 1990). In this respect, detailed studies have been made in some species, such as *Impatiens balsamina* (Nanda and Krishshnamoorthy 1967), *Citrus sinensis* (Lord and Eckard 1987) and *Anagallis arvensis* (Green *et al.* 1991). Reversion of flowers and inflorescences can be brought about by various treatments, such as high temperature, low irradiation, change between short days and long days and treatment with maleic hydrazide and glutamic acid (King 1983; Bettey and Lyndon 1990). In *W. bijieensis*, plants cultivated outdoors at low night temperature and low humidity have exhibited a high percentage of reversion in floral development. However, the reversion did not occur in the first group grown in controlled conditions similar to those of its natural habitat. The second and third group showed a small percentage of reversion in floral development. The comparison of the four experimental groups demonstrated that reversion of floral development is probably related to both low humidity and low night temperatures. The combination of these two ecological factors appeared to strengthen the reversion process.

Aberration occasionally occurs in the natural habitat. In the first group, aberrant flowers constituted only 5.6% of the flowers examined. In the second and third group, grown at low night temperatures or low humidity, the percentages of aberrant flowers were 11.1 and 6.7%, respectively. Thus, no remarkable differences exist among the first three experimental groups in respect to the formation of aberrant flowers. Nevertheless, in sharp contrast to the first three groups, 33.3% of the examined flowers were aberrant in the fourth group grown outdoors at low night temperature and low humidity. Moreover, aberrant plus reversed flowers constitute more than half of the total examined (58.3%) in the fourth group. Accordingly, the low night temperature or low humidity alone does not greatly affect the normal flowering process. Whereas the combination of these two ecological factors seems to considerably disturb the normal flowering process, while greatly increasing the reversion process of flowering. High humidity and moderate temperature, or at least one of the two, are necessary for the normal flowering of *W. bijieensis*.

As a series of discrete steps, the presence of the floral signal is required continuously, which comes from the progressive gene expression in interaction with environment (Bettey and Lyndon 1990). Floral reversion could be due to a failure to activate or maintain the expression of the flower meristem identity genes. The failure to maintain these genes in *Arabidopsis* can result in phenotypes reminiscent of floral reversion (Huala and Sussex 1992; Bowman *et al.* 1993; Okamuro *et al.* 1996, 1997). The evidence from higher plants shows that there are many potential points at which gene expression is controlled and many examples of gene reactivation can be found (Howell 1998). Development is a result of an interplay between gene action, developmental cues and environmental signals (Howell 1998). The end of the rainy season corresponds with the end of the flowering season of *Whytockia*. Changes in ecological factors at that time, such as low night temperatures and low humidity, are apparently signals affecting flowering, and thus gene expression, inducing a switch from floral meristem identity to vegetative. The observed aberrants and reversals could be seen as transitional organs, products of changes in the local climate from wet to dry season.

One additional phenomenon should be noted, namely the change of a floral primordium into a single bract-like organ. This has been observed both in the natural habitat during the late period of anthesis and in this experiment (Group 4). This indicates that it is not an isolated event, but could be interpreted as an extreme transition. This change has potential evolutionary implications, and indicates that a transition from pair-flowered to normal cymes can be achieved by a change from one flower to a bract-like organ in the flower pairs. Investigations on the genes involved in the flower development of *Whytockia* and their pattern of differential expression under diverse environmental conditions would be very revealing.

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