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Pollination Ecology of *Chloranthus serratus* (Thunb.) Roem. et Schult. and *Ch. fortunei* (A. Gray) Solms-Laub. (Chloranthaceae)

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Flowering and pollination biology of *Chloranthus serratus* and *Chloranthus fortunei* were studied. Flowering took place from early March to mid–April in *Ch. fortunei*, and from April to September, the whole growth period, in *Ch. serratus*. The flowering period of an inflorescence of *Ch. serratus* averaged about 8 d and anthesis of a single flower was 5–6 d. Flowers are slightly protogynous. The flower emitted fragrance when the androecium became white. Both species are entomophilous with thrips as exclusive pollinators. Under natural conditions, fruit set occurs mainly as a result of cross-pollination, but self-pollination and agamospermy may occur in some cases. In flowers of *Ch. fortunei* and *Ch. serratus*, the incurved androecium, the carpel and the spike axis form a nearly closed chamber that contains the anthers and stigma. The development of a floral-axial chamber may be an important step towards a more economical and effective pollination system. Floral morphology, pollination biology and fossil evidence suggest that the main evolutionary trend in the genus *Chloranthus* is towards development of 'closed' flowers. The fidelity of the relationship between *Chloranthus* and thrips is regarded as a specialized feature of pollination biology and this relationship may have originated early in the evolutionary lineage.

Key words: Chloranthaceae, *Chloranthus*, *Thysanoptera*, flowering, floral-axial chamber, pollination, evolution trends.

INTRODUCTION

The Chloranthaceae are regarded as one of the oldest extant families of angiosperms based upon extensive records of Cretaceous Chloranthaceae-like-pollen, flowers and vegetative parts (see Eklund, Friis and Pedensen, 1997). The extant family comprises four genera and about 75 species, and has a scattered distribution in East Asia, Malaysia, some western Pacific Islands, Madagascar and the Neotropics (Endress, 1994). The family can be divided into the *Chloranthus-Sarcandra* group and the *Ascarina-Hedyosmum* group (Endress, 1987). The former are shrubs or herbs with bisexual flowers, while the latter are trees or shrubs with unisexual flowers. The genus *Hedyosmum* is specialized for wind-pollination, while the genus *Ascarina*, at least in part, is also wind-pollinated but is less specialized than *Hedyosmum* (see Endress, 1994). On the other hand, *Chloranthus* and *Sarcandra* are thought to be entomophilous since they have conspicuous anthers with broad yellow or white connectives that produce scent, they produce a small amount of pollen and have a small and wet stigma (Endress, 1994). Moreover, Ma, Wang and Cui (1997) recently reported that *Chloranthus holostegius* is pollinated by thrips.

The genus *Chloranthus*, with an estimated 16–20 species, extends from Japan, China and eastern Russia to India, Sri Lanka, Malaysia and New Guinea (Wu, 1982; Verdcourt, 1986). The androecium varies greatly in form, but it is always three-lobed with swollen filaments and connectives. We selected three species representative of the different types of androecium in the genus. In *Ch. henryi* Hemsl. the androecium has three nearly completely separate lobes, in *Ch. serratus* the three short lobes are partly laterally-fused, and in *Ch. fortunei* the three laterally-fused lobes are extended as three linear white devices. *Ch. serratus* is widely distributed in south, east and central China, as well as in Japan. *Ch. fortunei* has a similar distribution to that of *Ch. serratus* but is absent in Japan, and *Ch. henryi* has a more western distribution than *Ch. fortunei*.

The present study describes the pollination biology of these species through extensive observation and behavioural analyses of floral visitors. The evolutionary state of the small, simple and bisexual flowers of *Chloranthus* is discussed from the view of pollination ecology. Evolutionary trends in the genus based upon the morphology of modern and fossil chloranthoid flowers and their pollination biology are suggested.

MATERIALS AND METHODS

Studies were carried out on two populations of *Ch. fortunei* (A and B), three populations of *Ch. serratus* (C, D and E) and one population of *C. henryi* (F) in Xinning County (26 23′ N, 110 31′ E), southwest, Hunan Province, China, about 10 km SE of the town of Jingshi. The populations are referred to by the above letters hereafter.

Observations on flowering phenology and reproductive systems extended from 13 March to 20 April. Complementary work was undertaken between 22 April and the end of October by a local botanist (Mr Luo Ling-bo). At the beginning of our investigation on 20 March, about one third of the individuals had already flowered in two populations of *Ch. fortunei*, so their early flowering was not recorded. In

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the three populations of *Ch. serratus*, leafy shoots had just emerged from the ground at this time. In the population of *Ch. henryi*, some individuals had just begun flowering, but its remote location hindered further observation. Therefore, the detailed observation and experiments were mainly based on the populations of *Ch. serratus* (C, D and E) and *Ch. fortunei* (A and B).

For cross- and self-pollination experiments, ten–15 inflorescences were isolated a few days prior to anthesis using semitransparent paper bags. The flowers were emasculated when their androecia became yellowish-green and the anthers from one inflorescence were kept together. The flowers were then pollinated by hand using pollen from different plants to effect cross-pollination and pollen from the same inflorescence for self-pollination. Three treatments were carried out prior to flowering: (1) emasculation, then isolation in bags; (2) emasculation, but no isolation; and (3) isolation without emasculation. Since flowers of *Ch. serratus* and *Ch. fortunei* are too small to handle easily, all flowers on one inflorescence were treated in a similar way. The fruiting spikes were collected 10–15 d after the anthers had abscised. In the case of mature fruits that had fallen from the axis of infructescences, we counted the bracts that had enlarged as a result of fruit maturation. Unfortunately, some of the individuals included in our treatments, and all of the treated individuals in population E were destroyed by animals and curious local people.

Flower visitors were observed in the field and voucher specimens were collected. Similar insects visiting other flowers near the populations of *Ch. serratus* and *Ch. fortunei* were also recorded and collected. The insects that appeared to be active pollinators were observed under a Hitachi S-800 scanning electron microscope (SEM). Pollen attached to the bodies of insects was identified against a reference collection of pollen from flowers in which the insects were collected. Voucher specimens were identified by entomologists at the Institute of Zoology, Chinese Academy of Sciences, and are deposited there. Voucher specimens of the *Chloranthus* species are kept in the Hebarium (PE), Institute of Botany, Chinese Academy of Sciences.

Data were analysed using analysis of variance and the means compared using the LSR test (or SSR test) (Ma, 1978).

RESULTS

Habit and habitat

The *Chloranthus* species investigated here are perennial herbs with subterranean rhizosomes and erect stems. Plants grow in aggregates and each plant has one–15 stems which develop from the same rhizome. Stems are 15–50 cm high in *Ch. serratus* and *Ch. fortunei*, and 40–65 cm high in *Ch. henryi*. Spikes are terminal, and there is only one in *Ch. fortunei*, two in *Ch. henryi* and one–three(five) in *Ch. serratus*.

All the species grow on acid soils and prefer disturbed habitats. *Ch. fortunei* grows on the N or NE slopes of mountains at elevations of approx. 450 m. The populations studied occurred along a path in sparsely artificial mixed deciduous evergreen plantations of *Cunnighamia lanceolata* (Lamb.) Hook. (Taxodiaceae) and *Vernicia fordii* (Hemsl.) Airy-Shaw (Euphorbiaceae). The biotopes varied in position from nearly open to heavily shaded under shrubs. *Ch. serratus* occurred on the northern slopes of mountains at elevations of approx. 500 m. It occupied two habitats: glades in plantations mainly consisting of *C. lanceolata* and *V. fordii*, and heavy shade within plantations of *C. lanceolata*. Both habitats were characterized by good drainage. *Ch. henryi* grew at the bottom of a deep gorge at about 600 m elevation under dense shrubs with good drainage.

Flowering season

The flowering season in *Ch. fortunei* was from early March to mid-April, and varied between individuals according to biotope. The time lag between the earliest and latest flowering plants in population A was about 1 month. Optimal blooming season, when most individuals were in flower, was from 10 to 25 March. Towards the end of the flowering season about 5% of individuals produced one to four new flowering shoots in the leaf axils. Those new flowering shoots bore two–four leaves and an inflorescence or bore only an inflorescence without leaves. The flowering season of the inflorescences on the new shoots lasted from 10–15 d.

New flowering shoots or new inflorescences were produced constantly throughout the growing period from April to September in *Ch. serratus*. The blooming season also varied greatly among individuals. The flowering periods of two successively produced new inflorescences were synchronized within the population as a whole, sometimes even within the single individual. The terminal primary inflorescence started flowering in mid-April and ended in mid-May. Secondary flowering shoots, produced in the leaf axils, opened in mid-May and lasted about 15 d. The secondary flowering shoots sometime bore two–three leaves. The tertiary inflorescences, produced at the leafless nodes below the secondary flowering

TABLE 1. *Number of flowers on spikes of* Chloranthus serratus *at different periods*

Type of inflorescence	No. of stems	No. of spikes	No. of flowers	No. of flowers per spike
Primary	12	17	290	17·0
Secondary	3	9	36	4·0
Tertiary	5	10	34	3·4
Quaternary	13	51	135	2·4
Fifth	6	26	60	2·3
Sixth	1	4	7	1·7

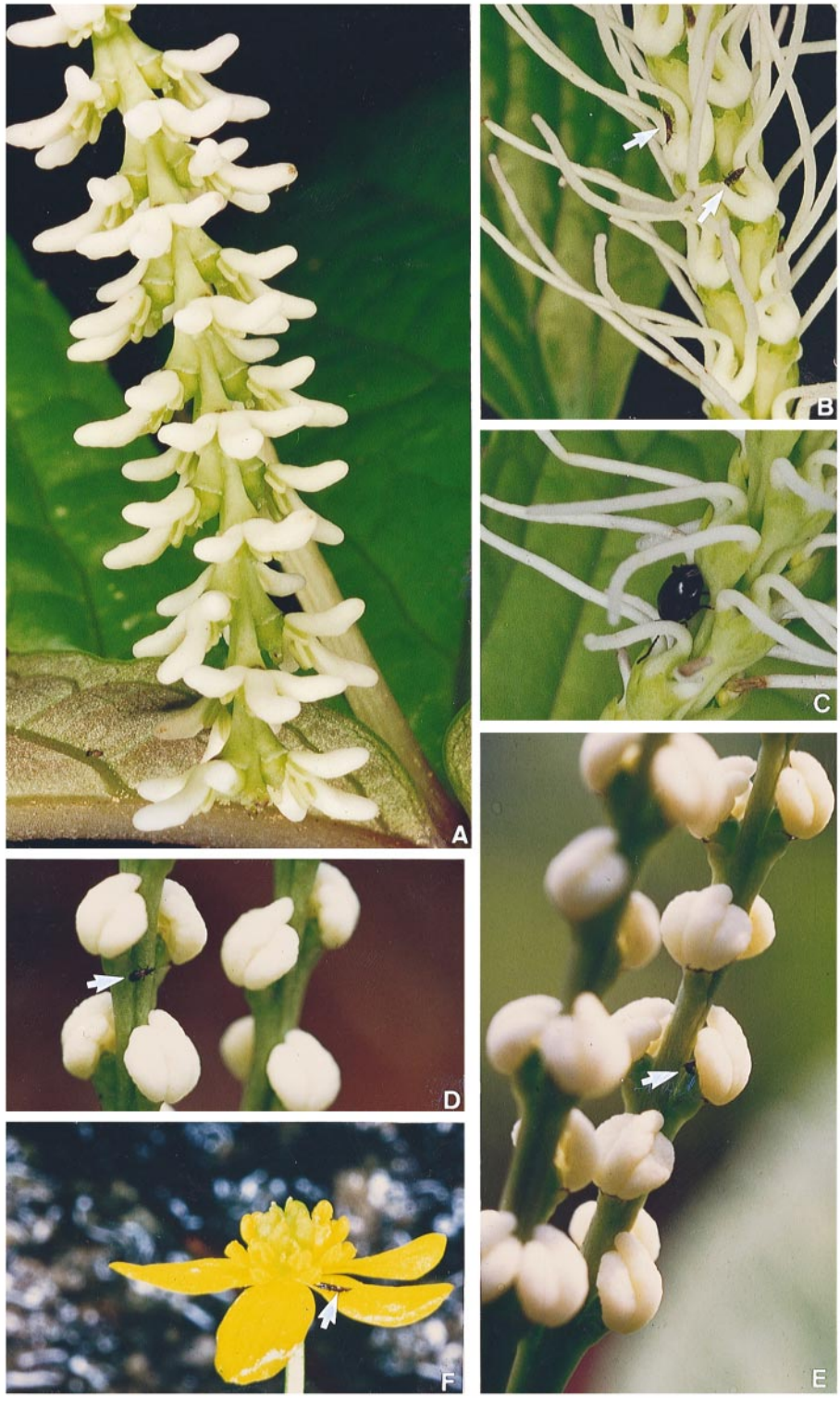

FIG. 1. A, *Chloranthus henryi* showing the inflorescences curving downwards at anthesis. ×5. B, *Ch. fortunei* showing two individuals of *Taeniothrips eucharii* (arrows) on the inflorescence. ×4. C, *Ch. fortunei*. The beetles are hindered by the appendages of the androecium. ×4. D, *Ch. serratus* showing *T. eucharii* (arrow) on the axis of the inflorescence. ×4. E, *Ch. serratus* showing *T. eucharii* (arrow) in the floral-axial chamber. ×4, F. *Ranunculus xinningensis* showing *T. eucharii* (arrow) with pollen grains attached to its body. ×4.

shoots, appeared in early June and ended late June. Subsequently, the following new inflorescences occurred repeatedly in the leaf axils or at the leafless nodes from mid-June to September. Similarly, in the leaf axils or leafless nodes of the secondary flowering shoot, new inflorescences were produced in the same order as on the stem. Generally,

TABLE 2. *Insects visiting* Chloranthus serratus *and* Ch. fortunei

Thyanoptera
Thripidae
Thaeniothrips eucaharii, 23 female, 1 male
Phlaeothripidae
Holothrips hunanensis (new sp.) 1 female
Haplothrips sp. (1 sp.), 3 female, 3 male
Coleoptera
Chrysomelidae
Lacticca sp. (1 sp.)
Luperomorpha sp. (1 sp.)
Calomicrus sp. (1 sp.)
Meloidae
1 sp. in larvae stage, 1 individual

except for the primary inflorescences, the stem produced new flowering shoots or inflorescences about four times, but sometimes seven to eight times. The secondary flowering shoots produced new inflorescences only up to three(–four) times. In the same leaf axil new inflorescences occurred two–three times and the late developing inflorescences always grew on the abaxial side of the early ones. New inflorescences usually occurred at the leafless nodes on the upper part of stem, but occasionally at the leafless nodes throughout the whole stem. The later an inflorescence appeared, the fewer flowers it bore (Table 1). Sometimes new inflorescences, especially those that occurred near the end of the growth period, produced incomplete flowers that only consisted of bracts.

Flower and fruit development

The primary inflorescence of *Ch. serratus* was enclosed by almost erect leaves when the stem emerged from the ground. These leaves appear to have a protective role during this period. Two or 3 d later, the leaves gradually spread and the primary inflorescence was exposed. The inflorescence consists of one–three(–five) spikes and each spike contained ten–25 flowers in decussate pairs or in an irregular order. All flowers were covered by green bracts. Within 1 week the top of the bract changed from green to brown and finally turned black. During this period, the bracts have an important protective function. Three to 4 d later, the green androecium expanded and was exposed. About 2 weeks later, the androecium became yellowish-green, and finally white. The anthers changed from green to yellow. At the same time, the gynoecium elongated and the stigma changed from grey to transparent white. The cavity of the stigma became filled with mucilaginous secretion, and the stigma became receptive. Scent production began synchronously, and a weakly fragrant odour became evident. After 1–2 d the androecium became white, and the anthers began to dehisce. Lateral thecae opened about 1 d before median thecae. The male phase extended over about 4 d, while the female phase lasted 2–3 d. The flower was slightly protogynous but the male and female phases overlapped slightly. The entire androecium fell off as a single unit about 2 d after the anthers had dehisced. The stigma became black when the androecium had fallen from the spike. The whole anthesis process of a single flower lasted about 5–6 d.

Flowers on the lower part of the spike changed colour earlier than those on the upper part, and the change in bract colour and the expansion of androecia followed the same order. However, the sequence of anther dehiscence differed: in individual spikes the lowermost, uppermost or even the middle flowers could open their anthers first, and the sequence in which the androecia fell was similar. The anthesis of a whole inflorescence averaged about 8 d. In *Ch. fortunei*, acropetal opening of flowers was observed in a single spike.

After about 1 week, the stigma became black and the carpels showed visible swelling. The entire process from blackening of the stigma to fruit ripening extended over 2 weeks. The ripened fruits fell from the axis within 10 d, and the axis then fell from the shoot. If no ripe fruits developed on the axis, the whole axis fell from the shoot about 1 week after the anthers had abscised.

Floral morphology

Details of floral morphology have been described previously for *Ch. serratus* and *Ch. fortunei* (Swamy, 1953; Endress, 1986). Therefore, only the floral structures that relate directly to pollination are described here. In *Ch. serratus*, when the androecium turns white the gynoecium elongates. The lengthening of the gynoecium causes the androecium to change from being tightly appressed to the spike axis to a situation in which it leans towards the axis and slightly covers the stigma (Fig. 1 D and E). The distance between the base of the androecium and the spike axis is about 1–1·5 mm, and the top of androecium almost touches the axis. On the other hand, the androecium is bell-shaped (involute). The bell-shaped androecium, together with the spike axis and the carpel, form an almost closed floral-axial chamber that contains the anthers and stigma (Fig. 1 E). There are two narrow entrances to the chamber from each side of the androecium. In *Ch. fortunei*, although the three appendages of the androecium bend backward, the floral-axial chamber is still formed. However, because the lateral lobes divide widely from the median one, two additional entrances are formed. In total, there are four entrances leading to the floral-axial chamber in *Ch. fortunei* (Fig. 1 B–C). In *Ch. henryi*, the entire androecium spreads from the spike axis and no floral-axial chamber is formed during anthesis (Fig. 1 A).

Flower visitors and pollinators

The number of different species of flower-visiting insects was quite small (Table 2). Only three species of *Thysanoptera* and three species of *Coleoptera* were observed visiting flowers of *Ch. fortunei* and *Ch. serratus*. The small thrips were regular visitors and were found on flowers nearly every day during our observation period. In both *Ch. serratus* and *Ch. fortunei*, larval stages and adults of *Thysanoptera* were found on the same inflorescence.

Female *Taeniothrips eucharii* Whetzel (Thripidae) were the predominant visitors to flowers of *Ch. fortunei*. Equal numbers of female and male individuals of a species of *Halothrips* and one female individual of *Holothrips hunanensis* Han (Phlaeothripidae) also visited the flowers of *Ch.*

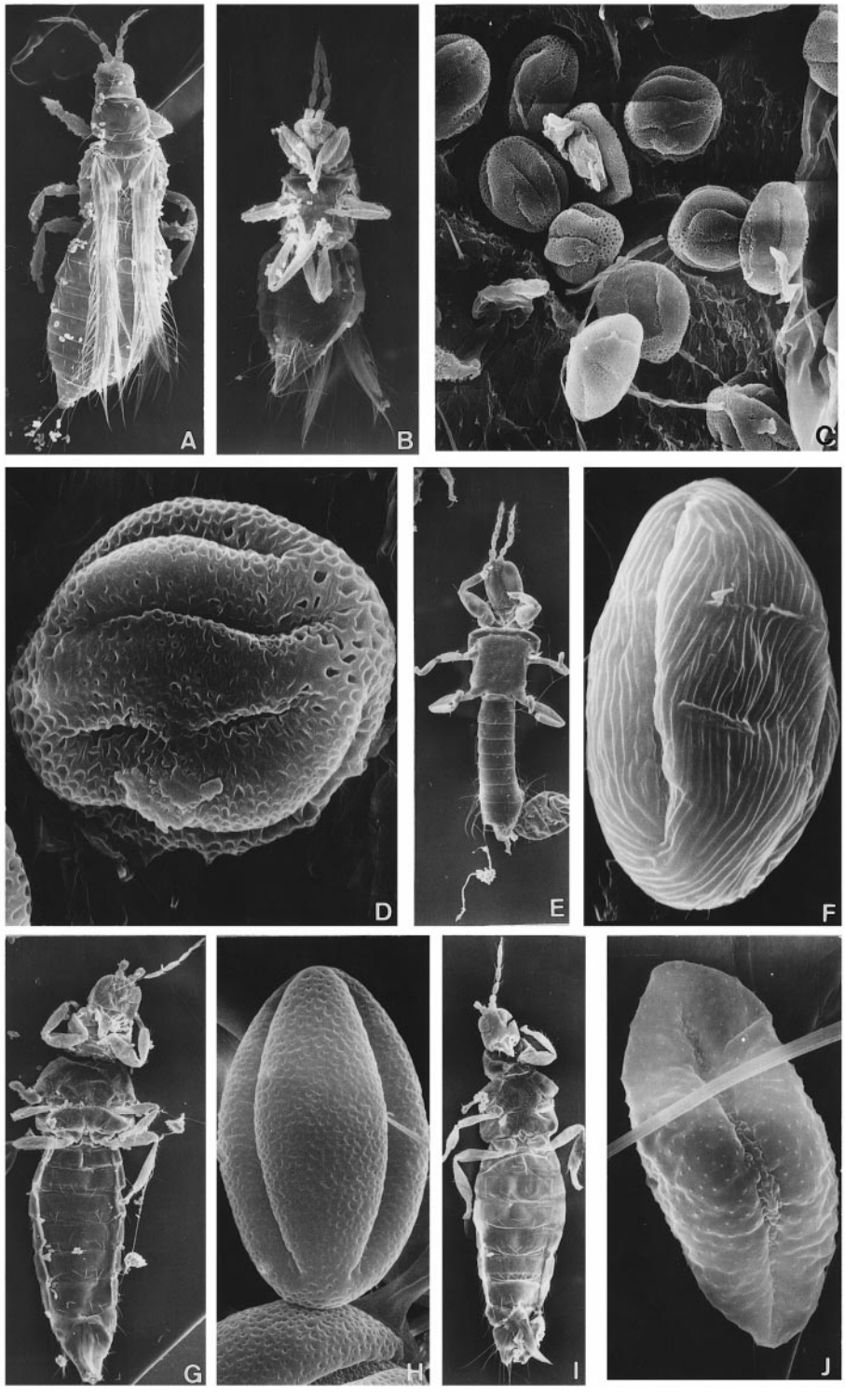

FIG. 2. A and B, SEMs showing dorsal and ventral views of *Taeniothrips eucharii* from the flowers of *Ch. serratus* showing pollen grains attached to the whole body. A, ×36. B, ×42. C, Detail of part of the body shown in B. Note the many pollen grains of *Ch. serratus*. ×600. D, Detail of pollen grain of *Ch. serratus*. ×2400. E, *Haplothrips* sp. from the flowers of *Potentilla kleiniana*. ×30. F, Detail of pollen grain of *P. kleiniana*. ×2100. G, *T. eucharii* from the flower of *Sargentodoxa cuneata*. ×36. H, Detail of pollen grain of *S. cuneata*. ×2100. I, *T. eucharii* from the flower of *Ranunculus xinningensis*. ×30. J, Detail of pollen grain of *R. xinningensis*. ×2100.

fortunei. However, only the female *T. eucharii* visited flowers of *Ch. serratus* during our observation periods (Fig. 2A–D).

In the study areas of *Ch. fortunei*, Chrysomelidae beetles were mainly present on flowers of *Sargentodoxa cuneata* (Oliv.) Rehd. et Wils. (Sargentodoxaceae) and *Zanthoxylum*

dimorphophyllum Hemsl. (Rutaceae), and while they occasionally visited flowers of *Ch. fortunei* they appeared too large to enter its floral-axial chamber; the beetles were blocked by the appendages of the androecium (Fig. 1C). Because these beetles do not have elongated mouthparts that could probe into the cavity they can be dismissed as pollen vectors of *Ch. fortunei*. Small spiders were frequently found on the inflorescences of *Ch. fortunei* and *Ch. serratus*. They were predatory on both adult and larval thrips.

Phenology of pollinators and their visits

In populations of *Ch. fortunei*, two species of thrips were frequently found on at least three other plant species, such as *Potentilla kleiniana* Wright et Arn. (Rosaceae), *Ranunculus japonicus* Thunb. (Ranunculaceae) and *Sargentodoxa cuneata*. The flowering seasons of these plants were somewhat later than those of *Ch. fortunei*. In populations of *Ch. serratus*, only *Ranunculus xinningensis* W. T. Wang was visited by thrips in early April, before the flowering season of *Ch. serratus* (Fig. 1F).

Under the SEM, it is easy to distinguish pollen grains of *Chloranthus* from those of *Sargentodoxa cuneata*, *Potentilla kleiniana* and *Ranunculus xinningensis* by their aperture and ornamentation features (Fig. 2D, F, H and J). Our observations showed that the pollen grains attached to the bodies of thrips were completely consistent with the flowers they visited (Fig. 2A–J). Moreover, under SEM, we checked four thrips which had visited flowers of *Chloranthus* and found only *Chloranthus* pollen grains. Obviously, during their period of anthesis the two species of *Chloranthus* are the only or main food source for the thrips in the study area.

Generally, thrips are short-lived and can build up large populations within a short time (Endress, 1994). Appanah and Chan (1981) reported that thrips complete their life cycle, from egg to adult, within 8 d at a temperature of 22–32 °C. During the main flowering season of *Ch. fortunei* and *Ch. serratus*, the temperature range in our study areas was 5–19 °C in March, 14–27 °C in April and 20–34 °C in May according to local weather station records. Therefore, it is possible that the life cycle of thrips in our study area was similar to that reported by Appanah and Chan (1981). At least we can speculate that at the population level, more than one generation of thrips appeared on the flowers of *Ch. serratus* and *Ch. fortunei* because the flowering season for these two species varied greatly among individuals. At the individual level, however, thrips can go through their entire life-cycle in the flowers of one inflorescence since anthesis of one inflorescence of *Ch. serratus* and *Ch. fortunei* lasts about 8 d. The continuous availability of floral resources to thrips facilitates the build up of their populations throughout the whole flowering season of *Chloranthus*. Based on thrip pollination in some species of Compositae, Ananthakrishan (1982) suggested that the continuous build up of pollinating thrip populations in a flower can lead to migration of thrips, thus enhancing the possibilities of cross-pollination. Apparently, a similar mechanism to enhance the occurrence of cross-pollination also appears in these two species of *Chloranthus*.

Pollinator behaviour

As the androecium turned yellowish-green or white, and the flower emitted a weakly fragment odour, thrips began to appear on the inflorescences; most of them were black adults. Obviously, these adult thrips carried pollen grains from other individuals if pollen grains were present on their bodies. When the first group of thrips arrived on the inflorescences, the anthers had yet to dehisce. But the entrances to the floral-axial chamber had formed and the stigma was receptive. Thrips were distributed over the whole inflorescence including the axis, the dorsal surface of the androecium, the appendages of the androecium, and the floral-axial chamber (Fig. 1D and E). They moved quickly from flower to flower, crawled into and out of floral-axial chambers and sometimes remained inside for a short time (less then 3 min). Sometimes they gnawed the unopened anthers. During this period, the activities of the adult thrips mainly resulted in cross-pollination.

The number of insects increased as anthesis proceeded and the fragrant odour strengthened, reaching a peak when most of the anthers had dehisced. However, once the anthers had opened, thrips mainly stayed in the floral-axial chambers (Fig. 1E) and only occasionally moved about on the inflorescence axis. While feeding on the opened anthers, thrips may deposit pollen on the stigma. This may favour self-pollination. Towards the end of anthesis, the adult animals moved away, but the larvae stayed on the inflorescences until the androecia had fallen from the axis.

On sunny days thrips were present on the inflorescences from early morning (about 0630 h) to evening (about 1700 h). During the night they hid in floral-axial chambers or moved away. On rainy days they remained in floral-axial chambers. In their larval stage thrips are wingless and remain on the same inflorescence until they develop into adults. Adult thrips have wings and are able to fly, but direct observations of their approach to flowers are still lacking. Direct examination of spikes showed that there were one to six individuals on a spike during anthesis of the spike.

Both larval and adult thrips were sensitive to disturbance, such as slight shaking of the inflorescence or blowing on it. During early anthesis, the adult animals quickly moved towards the base of the inflorescence following disturbance and hid in the furrows of petioles, leaf axils or the dorsal surface of leaves, while the larvae moved towards the upper part of the inflorescence and hid in the floral-axial chamber. Ten to 15 min after the disturbance, the adult animals returned to the inflorescence, but later they moved on, thus increasing the possibility of outbreeding.

Therefore, thrips mainly cause cross-pollination of *Ch. fortunei* and *Ch. serratus*, but occasionally self-pollination may occur based on phenological and behavioural data of thrips.

Fruit set and mating systems

Under natural pollination conditions, about 90 and 50% of the individuals of *Ch. serratus* (population C) and *Ch. fortunei* (population A), respectively, shed their primary

TABLE 3. *Fruit set after different treatments of the primary inflorescences of* Ch. fortunei (*populations A and B*)

Treatment	No. of stems	No. of flowers	No. of mature fruit	Fruit set %		
				Mean	s.d.	Range
Isolated without emasculation	4	60	34	59·76	0·30	0·00–0·86
Emasculated with isolation	12	218	23	11·51	0·14	0·00–0·71
Emasculated without isolation	6	115	10	8·75	0·12	0·00–0·40
Manual self-pollination	2	32	5	14·50	0·11	0·07–0·22
Manual cross-pollination	7	132	112	87·61	0·11	0·69–1·00

TABLE 4. *Fruit set after different treatments of the primary inflorescences of* Ch. serratus (*populations C and D*)

Treatment	No. of stems	No. of flowers	No. of mature fruit	Fruit set %		
				Mean	s.d.	Range
Isolated without emasculation	14	315	48	9·57	0·13	0·00–0·55
Emasculated with isolation	12	332	1	0·56	0·10	0·00–0·07
Emasculated without isolation	12	385	5	1·55	0·03	0·00–0·11
Manual self-pollination	10	200	17	10·41	0·12	0·00–0·44
Manual cross-pollination	15	382	94	25·37	0·23	0·00–0·86

TABLE 5. *Fruit set after different treatments of the primary inflorescences of* Ch. fortunei (*populations A and B*), *excluding inflorescences that were shed and therefore had no mature fruit*

Treatment	No. of stems	No. of flowers	No. of mature fruit	Fruit set %		
				Mean	s.d.	Range
Isolated without emasculation	3	42	34	78·3	0·07	0·70–0·86
Emasculated with isolation	6	108	23	23·0	0·18	0·05–0·71
Emasculated without isolation	2	36	10	18·7	0·06	0·13–0·25
Manual self-pollination	2	32	5	14·5	0·11	0·07–0·22
Manual cross-pollination	7	132	112	87·6	0·11	0·69–1·00
Natural pollination	128	2560	2017	76·1	0·22	0·08–1·00

inflorescences before the fruits had matured. Similarly, some experimentally-treated inflorescences fell from stems before their fruits had matured. The results of fruit set in the five treatments are given in Table 3 and 4. To compare fruiting in our five treatments with that under natural pollination, we excluded inflorescences that shed before fruits became mature from Table 3 and 4, creating two new tables including fruit set under natural pollination (Tables 5 and 6).

We compared the fruit set of flowers that were manually self- or cross-pollinated, emasculated with or without isolation, and isolated without emasculation to test whether: (1) flowers are pollinated by insects or are autonomous self-pollinaters; or (2) flowers are self-pollinated or cross-pollinated. Differences in fruit set among treatments were significant in *Ch. fortunei* ($F = 46{\cdot}93$, d.f. = 30, $P < 0{\cdot}01$, Table 3; $F = 19{\cdot}24$, d.f. = 22, $P < 0{\cdot}01$, Table 5) and *Ch. serratus* ($F = 4{\cdot}65$, d.f. = 62, $P < 0{\cdot}01$, Table 4; $F = 10{\cdot}28$, d.f. = 51, $P < 0{\cdot}01$, Table 6).

In *Ch. fortunei*, the fruit set of flowers that were manually cross-pollinated (87·6%) and those that were isolated without emasculation (59·76%) was significantly higher than that of those manually self-pollinated or emasculated with or without isolation (8·75–14·50%) ($LSR_{\alpha=0{\cdot}01} = 25{\cdot}51$–$27{\cdot}91 < 43{\cdot}35$–$50{\cdot}92$). In addition, the fruit set of manually cross-pollinated flowers was higher than that of flowers isolated without emasculation ($LDR_{\alpha=0{\cdot}01} = 25{\cdot}51 < 27{\cdot}82$) (Table 3). However, treatments involving manual self-pollination and emasculated with or without isolation showed no significant differences in fruit set (Table 3). In Table 5, results of fruit set were basically similar to those in Table 3 except that fruit set showed no significant difference irrespective of whether flowers were pollinated naturally, manually cross-pollinated or isolated without emasculation. Thus, it is possible that the relatively high fruit set in flowers which were isolated without emasculation resulted because isolation occurred too late to prevent the thrips from visiting, rather than by autonomous self-pollination. Given the above results, we suggest that cross-pollination, self-pollination and agamospermy can all contribute to fruit set in *Ch. fortunei*, but that cross-pollination is the largest contributor under natural conditions.

In *Ch. serratus*, the fruit set of manual cross-pollination (25·37%) was significantly higher than that of flowers which were emasculated with or without isolation ($LSR_{\alpha=0{\cdot}05} = 15{\cdot}18$–$15{\cdot}48 < 17{\cdot}43$–$18{\cdot}43$ (Table 4). On the other hand, fruit set did not differ significantly between treatments i.e. self-pollination, isolation without emasculation, and emas-

TABLE 6. *Fruit set after different treatments of the primary inflorescences of* Ch. serratus (*populations C, D and E*), *excluding inflorescences that were shed and therefore had no mature fruit*

Treatment	No. of stems	No. of flowers	No. of mature fruit	Fruit set %		
				Mean	s.d.	Range
Isolated without emasculation	5	162	48	26·8	0·21	0·03–0·55
Emasculated with isolation	1	15	1	6·6	—	—
Emasculated without isolation	2	58	5	9·3	0·02	0·07–0·11
Manual self-pollination	5	94	17	20·6	0·16	0·03–0·44
Manual cross-pollination	9	224	94	42·4	0·25	0·15–0·86
Natural pollination C	20	863	615	66·9	0·19	0·05–1·00
D	30	1250	823	69·3	0·29	0·11–1·00
E	16	750	490	72·9	0·21	0·20–1·00

TABLE 7. *Relative frequency of the number of mature fruit produced in infructescences of* Chloranthus fortunei (*populations A and B*)

Treatment	No. of infructescences examined	Percent of mature fruit in infructescences		
		> 0 and $\leqslant 50$	> 50 and < 100	100
Isolated without emasculation	3	0	3	0
Emasculated with isolation	6	3	3	0
Emasculated without isolation	2	2	0	0
Manual self-pollination	2	2	0	0
Manual cross-pollination	7	0	5	2
Natural pollination	130	22	101	7

TABLE 8. *Relative frequency of the number of mature fruit produced in infructescences of* Ch. serratus (*populations C and D*)

Treatment	No. of infructescences examined	Percent of mature fruit in infructescences		
		> 0 and $\leqslant 50$	> 50 and < 100	100
Isolated without emasculation	5	4	1	0
Emasculated with isolation	1	1	0	0
Emasculated without isolation	2	2	0	0
Manual self-pollination	5	5	0	0
Manual cross-pollination	9	6	3	0
Natural pollination	109	19	73	17

culation, with or without isolation. However, fruit set of flowers following natural pollination (66·9–72·9 %) was significantly higher than that of flowers subjected to the other five treatments ($LSR_{\alpha=0\cdot05} = 22\cdot71–26\cdot20 < 25\cdot06–70\cdot47$), and fruit set did not differ significantly among those five treatments (Table 6). These results reflect the fact that cross- and self-pollination and agamospermy may coexist in *Ch. serratus*, and that cross-pollination contributes substantially to fruit set relative to agamospermy, while it is difficult to discriminate between the contribution to fruit set made by cross- and self-pollination under natural conditions.

Moreover, the relative frequencies of mature fruits in infructescences in both *Ch. fortunei* and *Ch. serratus* under natural pollination conditions show that only occasionally can all flowers on an inflorescence develop into mature fruits (Tables 7 and 8). On the other hand, under natural conditions the fruit sets of both species varied greatly among individuals within one population (Tables 5 and 6). Therefore, extensive agamospermy and autonomous self-pollination do not appear to occur in *Ch. fortunei* and *Ch. serratus*.

DISCUSSION

Floral form and function

Since the flowers of *Chloranthus* lack a perianth, the androecium has taken over some of its functions. Endress (1987) suggested that the androecium in some *Chloranthus* species has three functions (apart from pollen production): as an osmophore, a semaphore, and in protection, while in some species (e.g. *Ch. fortunei*, *Ch. japonicus* Sieb. and *Ch. angustifolius* Oliv.) the androecium does not cover the gynoecium at anthesis, and has no protective function.

However, our observations show that in *Ch. fortunei* the gynoecium is still covered and protected by the coherent basal part of the androecium during the entire process of anthesis. Only three terete white appendages of the androecium have curved into a horizontal position from the inflorescence axis (Fig. 1C). We observed that the gynoecium in *Ch. henryi* is exposed at anthesis (Fig. 1A), but because its entire inflorescence curves downwards from the base of the spike during anthesis, the androecium still has a protective function. In *Ch. spicata* (Tunb.) Makino and *Ch. erectus* Sweet the adjacent lobes have nearly completely cohered and the androecium has reached such a developed stage as to almost completely envelope the distal half of the pistil. Therefore the androecium in all species of *Chloranthus* has a more or less protective function, especially in those species whose flowers have a floral-axial chamber. The formation of the floral-axial chamber has brought the pollen sacs and pistil into the same space. In other words, a flower with a floral-axial chamber is comparable to a closed flower, while flowers without it are comparable to open flowers.

As a result of the formation of the floral-axial chamber and the androecium coming to lie over the gynoecium in most species of *Chloranthus*, autonomous self-pollination can occur. However, it seems there is a mechanism to avoid autonomous self-pollination through the reduction and sterility of the anthers on the median lobe, or even their total absence. Species such as *Ch. angustifolius* and *Ch. japonicus* show features typical of this mechanism. In the species studied here, as well as in other species, the anthers on the median lobe are normal and functional. In these cases autonomous self-pollination seems to be avoided through slight protogyny. Ma, Wang and Cui (1997) also suggested that in *Ch. holostegius* (Hand.-Mazz.) Pei et Shan inbreeding occurs, and self-pollination does not exist under natural conditions. *Chloranthus* species which lack a floral-axial chamber in their flowers, and have anthers located on the base of the median lobe or even covering the entire median lobe, also seem to have mechanisms to avoid autonomous self-pollination. For example, the whole inflorescence of *Ch. henryi* curves downwards from the base of the axis and finally becomes pendulous or catkin-like during anthesis (Fig. 1A). Thus, pollen from the median anthers cannot fall directly onto the stigma, and thus autonomous self-pollination is avoided. In *Ch. oldhami* Solms-Laub., endemic to Taiwan, the flower is unique in possessing a long style (about 0·7 mm) which positions the stigma almost above the thecae, thereby avoiding autonomous self-pollination. Autonomous self-pollination is therefore avoided in different ways in the genus *Chloranthus*. However, self-pollination by thrips may occur to some degree, at least in *Ch. serratus*.

Pollination biology

Considering the optical and olfactory effects resulting from the broadened and sometimes elongated androecium within the family Chloranthaceae, Endress (1986) hypothesized that a cantharophilous pattern has evolved independently in an unusual way during its adaptive radiation. However, at least in *Sarcandra chloranthoides* Gardn., some features such as the minute thecae with low pollen content and the small and wet stigma, are not features of typical cantharophily (Endress, 1987).

Our field observations showed that, at least in *Ch. fortunei* and *Ch. serratus*, the most abundant insects on inflorescences are thrips, while beetles are only occasionally observed. SEM observations have further demonstrated that thrips can be effective pollinators because they carry a large pollen load. Ma *et al.* (1997) also reported that the pollinator of *Ch. holostegius* is a species of Thripidae. *Ch. fortunei* and *Ch. serratus* have some features similar to *Sarcandra chloranthoides* but their stamens are smaller, less conspicuous and less scented. Generally, in the genus *Chloranthus*, some species (e.g. *Ch. spicata* and *Ch. erectus*) can emit a strong odour, but their androecium is very small. Other species (e.g. *Ch. fortunei*, *Ch. japonicus*, *Ch. angustifolius* and *Ch. holostegius*) have a conspicuous androecium but are less scented. In addition, the number of flowers in an inflorescence in *Chloranthus* is higher than that in *Sarcandra chloranthoides*. Therefore, the genus *Chloranthus* shows none of the typical cantharophilous features as suggested by Gottsberger (1988).

Endress (1994) summarized the features of flowers adapted to pollination by thrips and suggested that flowers that provide shelter together with narrow entrances towards the floral centre are favoured by thrips. In addition, scent and white flowers are attractive to thrips, especially when combined. Nevertheless, flowers adapted to pollination by thrips share some features with flowers pollinated by small beetles. In *Chloranthus*, flowers have conspicuous anthers with broad yellow or white connectives that produce scent. Most species of *Chloranthus*, as well as *Ch. serratus* and *Ch. fortunei*, have a floral-axial chamber that could provide a favourable microenvironment for thrips.

Based on present knowledge, pollination by thrips appears in only five families of the Magnoliales and Laurales: Winteraceae, Annonaceae, Myristicaceae, Monimiaceae and Lauraceae (Endress, 1986, 1990). The Winteraceae have a broad array of pollinators, such as beetles, moths and thrips (Pellmyr *et al.*, 1990). In the genus *Belliolum*, thrips are an exclusive or primarily pollinator, while they act as co-pollinators in *Drimys* and *Pseudowintera* (Winteraceae) (Thien, 1980; Bernhardt and Thien, 1987; Pellmyr *et al.*, 1990). The family Annonaceae illustrates different degrees of adaptation to beetle pollination, and only a few species (e.g. *Xylopia aromatica*) have flowers pollinated by thrips as a co-pollinator (Gottsberger, 1988, 1989). Thrips are also co-pollinators in Lauraceae (Endress, 1990). One species of *Comproneura* (Myristicaceae) and one species of *Mollinedia* (Monimiaceae) both of which have unisexual flowers, are pollinated by thrips (Gottsberger, 1977; Bawa *et al.*, 1985).

The genus *Belliolum* has several pollination features similar to those of *Chloranthus*, as well as some obvious differences in floral structure (Thien, 1980; Pellmyr *et al.*, 1990). It is clear that, as noted by Thien (1980), *Belliolum* is pollinated by thrips but not because of a lack of larger insects and/or weather conditions prohibiting pollination by other insects. After analysing the floral fragrance

composition in four genera and twelve species of Winteraceae, Pellmyr *et al*. (1990) suggested that one species of *Belliolum* has an exclusive fragrance that consists almost entirely of linalool and linalool oxide, and this may be the reason this genus depends on thrips for pollination. Some Monimiaceae have specialized in respect to pollination by Thysanoptera (see Gottsberger, Silberbauer-Gottsberger and Ehrendorfer, 1980). According to Endress (1980), extremely specialized flowers have occurred in at least some genera of Monimiaceae. From the examples mentioned above, it appears that exclusive pollination by thrips is always connected with flowers that have a specialized floral structure or floral fragrance. It remains unclear whether the chemical composition of the floral fragrance is specialized in some species of the genus *Chloranthus*. However, it is possible that the apparently faithful association between *Chloranthus* and *Thysanoptera* is specialized within the evolutionary lineage of this genus.

Evolutionary trends in the genus Chloranthus

Based on the degree of lateral fusion of stamen lobes and the position of anthers on the medial lobe, Swamy (1953) recognized four types of floral structure in *Chloranthus*. However, Wang, Huang and Wu (1984) suggested that the lateral lobes, being reduced or absent in flowers of *Ch. multistachys*, represent only a form of *Ch. henryi* in later flowering periods. Indeed *Ch. multistachys* is now considered conspecific with *Ch. henryi*. The third type of flower recognized by Swamy (1953) should apparently be placed into the first type with *Ch. henryi*, if the observations of Wang *et al*. (1984) are correct. Our morphological, observations in *Ch. henryi*, *Ch. serratus* and *Ch. fortunei* confirm, respectively, Swamy's (1953) three types of flower.

The morphology of fossil chloranthoid androecia from the Early and Late Cretaceous indicate that the androecium in extant *Chloranthus* has arisen by fusion and other modifications of three separate and normal stamens (Friis, Crane and Pedersen, 1986; Crane, Friis and Pedersen, 1989; Herendeen, Crept and Nixon, 1993; Eklund *et al*., 1997). Free stamens may thus be an ancestral state for the genus *Chloranthus* (Eklund *et al*., 1997). Up to now, at least three distinct species of *Chloranthistemon*, e.g. *C. alatus* Eklund, Friis et Pedersen, *C. endressii* Crane, Friis et Pedersen and *C. crossmanensis* Herendeen, Crept et Nixon are known to have been present in the Late Cretaceous flora (Herendeen *et al*., 1993; Eklund *et al*., 1997). In *C. alatus* the apical sterile tissues of the stamen form conspicuous wing-like structures that are folded over the upper part of the adaxial surface. Meanwhile, there is a wing-like structure on each margin of the median stamen and the outer margin of lateral stamens. In *C. endressii*, the apical connectives of the stamens cohere to form a massive shield that is slightly adaxially reflexed (Eklund *et al*., 1997), while in *C. crossmanensis* the stamens with a triangular structure at the apical part are more or less curved (Herendeen *et al*., 1993). The features of the stamens of *C. alatus* and *C. endressii* suggest that these plants are entomophilous (Eklund *et al*., 1997). Obviously, all of these species have a more or less 'closed' structure in the flower that is similar to that of extant species. Furthermore, Friis *et al*. (1986) suggested that changes relating to the loss of two pollen sacs in each of the lateral stamens, and the shift to adaxially positioned pollen sacs, may have occurred as part of a trend towards increasing the efficiency of pollination.

Considering pollination data of the two *Chloranthus* species described above, formation of a floral-axial chamber is an adaptation to pollination by a certain group of insects. Combined with the morphological information from macrofossils, we suggest that the main evolutionary trend in the genus *Chloranthus* is towards development of a closed flower. Accompanying this development, a special and efficient pollination system appears to have developed in some species of *Chloranthus*.

Based on megafossil evidence, Herendeen *et al*. (1993) suggested that the modern genus *Chloranthus* had differentiated by the Turonian. It is possible that the Turonian types of stamens are a basal form, marking the minimal divergence time of modern Chloranthaceae from earlier chloranthoid ancestors (Crept and Nixon, 1994). In particular, two specimens of *Chloranthiestemon* from the Late Cretaceous that include well-preserved fragments of inflorescence axes with flowers *in situ*, described by Eklund *et al*., 1997, further document that chloranthaceous plants were well established by the Late Cretaceous. Interestingly, thrips are an ancient group of insects that are represented in the geological record prior to the Cretaceous (see Thien, 1980). Meanwhile, at least one group of beetles, the Curculionidae, were diverse by the Turonian (see Crept and Nixon, 1994). Generally, curculionid beetles are small in size. These tiny beetles are exclusive pollinators of some modern tropical flowers, such as Eupomatiaceae and Degeneriaceae (Thien, 1980; Armstrong and Irvine, 1990). However, thrips, rather than Curculionidae are the exclusive pollinators of some species of *Chloranthus*. Therefore we speculate that the association between *Chloranthus* and thrips perhaps developed very early, at least during the Turonian or Late Santonian/Early Campanian periods. Because of the absence of a modern phylogenetic analysis of the thrips, and their very scarce fossil record (see Pellmyr *et al*., 1990), we cannot speculate which group of thrips were associated with *Chloranthus* during the Cretaceous period nor if the extant association between *Thaeniothrips* and *Chloranthus* is ancient or more recent. In any case, pollination by thrips of the extant primitive *Chloranthus* described above has confirmed the suggestion of Thien (1980) that the extant primitive angiosperms exhibit diversified modes of pollination. Cantharophily cannot be constructed as the only mode of pollination among early angiosperms despite it being the most widespread form of pollination in extant magnoliale angiosperms.

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