A preliminary study on pollination biology of three species in *Dioscorea* (Dioscoreaceae)

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Abstract: *Dioscorea* (Dioscoreaceae), although a monocot, has many dicotyledonous features and plays a special role in the evolution of angiosperms. Because of a general lack of studies concerning aspects of pollination biology in this genus, three representative species, *D. zingiberensis*. *D. polystachya* and *D. bulbifera*, were investigated to remedy this deficiency. In this study, two repetitive observations of sex ratio and distribution, floral biology, pollination, seed germination of three representative species were studied. *D. zingiberensis* reproduces sexually with low seed germination rate. *D. polystachya* reproduces by bulbils on Mt. Wudang and by seeds at Yingxiu. All plants of *D. bulbifera* at Yingxiu grew from bulbils, but a small percentage of flowers at Mayidui were wind pollinated. The results indicate a possibility of wind pollination in *D. zingiberensis*. However, *D. polystachya* is pollinated by thrips. *D. bulbifera* is wind pollinated in regions where staminate and pistillate plants are intermixed. We deduced that the evolutionary trend in the flowers of *Dioscorea* ranges from totally open to half closed to nearly closed. And the sexual reproduction of *Dioscorea* species is obstructed.
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1. Introduction

On the basis of the floral features, Dioscoreaceae is assumed to be insect pollinated (entomophilous), and possibly by nocturnal insects (Burkill 1960). The pollinators of *Dioscorea spiculiflora* in sect. *Apodostemon* were found to be flies and ants (Martin *et al.* 1963). Pollinators of *D. nipponica* subsp. *rosthornii* in sect. *Stenophora* were species of *Halictus* and *Andrena* (Zhao *et al.* 2008). Thrips were reported to pollinate *D. rotundata*, *D. alata* and *D. japonica* in sect. *Enantiophyllum* (Martin *et al.* 1963; Pitkin 1973; Abraham and Nair 1990; Segnou *et al.* 1992; Bournier 1994; Mizuki *et al.* 2005). In addition, some species with high natural fruit set, such as *D. composita* and *D. floribunda* in sect. *Heterostemon*, have been proven to be wind pollinated (anemophilous). The pollen of these species can be found in air and fruit set is closely linked to the distances between individuals (Martin *et al.* 1963).

Dioscorea plays a very special role in the evolution of angiosperms, because it is a monocot with many dicot features. *Dioscorea* reproduces not only by seed, but also by rhizomes, tubers and bulbils. Species in different sections vary significantly in their reproductive characteristics. Such as the species in sect. *Stenophora* have primitive features and rhizomes, yellowish green, light yellow or orangish yellow fragrant flowers, or purple or aubergine widely open flowers with no fragrance. Species in section *Enantiophyllum*, with more advanced features, has straight tubers and white, light green or light yellow fragrant flowers that are nearly closed, and abundant bulbils. Sect. *Opsophyton* has features connecting sections *Stenophora* and *Enantiophyllum*. The species have globose tubers, and partially open white, pink or purple flowers (Burkill 1960; Pei and Ting 1985; Ting and Michael 2000).

The pollination and reproductive biology of *Dioscorea* is complicated and interesting due to the unique and varied floral morphology, but in fact, there is a lack of studies concerning these aspects, especially for species distributed in the Himalaya-Hengduan Mountains region where Dioscoreaceae is believed to have originated (Wan *et al.* 1994). The pollination biology of three species of *Dioscorea* in Hubei province, China has been taken in previous study (Zhao *et al.* 2007), but without discussing the evolutionary pattern in *Dioscorea*. Therefore, we conducted an extensive study of sex ratios and distribution, floral biology, pollination and seed germination of three representative species, *D. zingiberensis* (sect. *Stenophora*), endemic to China, *D. polystachya* (sect. *Enantiophyllum*) and *D. bulbifera* (sect. *Opsophyton*) to better understand the pollination and reproductive biology of *Dioscorea*, as well as the evolutionary pattern.

2. Material and Methods

Study species and areas

This study took place at Mt. Wudang (Danjiangkou city, Hubei Province, 32°23′ - 32°33′ N, 110°57′- 110°14′ E) for *D. zingiberensis* and *D.*

polystachya, Yingxiu (Dujiangyan city, Sichuan Province, 30°45′-31°43′N, 102° 51′-103°44′E) for *D. bulbifera* and *D. polystachya*, and Mayidui (Lincang city, Yunnan Province, 30°4′5-31°43′N) for *D. bulbifera*.

Two repetitive observations each on wild growing plants of *D. zingiberensis*, *D. polystachya* and *D. bulbifera* were made when the plants were at full anthesis from mid June to mid July in 2008 and 2009.

Sex ratio and distribution

Staminate and pistillate plants at the above sites were marked at their full bloom stage and the distances between them were measured.

Floral biology

Number of flowers and inflorescences, and blooming duration

Ten each of staminate and pistillate individuals of three species were randomly selected. The number of inflorescences and number of flowers per inflorescence were counted for each. Ten inflorescences were randomly chosen to record the blooming duration of the inflorescence and flowers. Blooming duration of the inflorescence was recorded from the opening of the first flower to the wilting of the last flower. The blooming duration of each flower was recorded from the first opening of the bud to wilting of the flower.

Pollen quantity per flower

Ten staminate buds were fixed in FAA solution (formalin-acetic acid-alcohol = 5: 5: 90). The anther walls were softened using 1.0 M HCl. The anthers were then cut and the pollen was transferred to a graduated centrifuge tube and distilled water was added until the total volume of the solution was 1 mL. The suspension was shaken for 60 s. Small amounts of liquid were drawn to count the number of pollen grains under a light microscope (Wang *et al.* 2005).

Structure of nectary

Measurements were taken of 15 each staminate and pistillate flower. The diameter of the tepals and open flowers were measured with the aid of an anatomical lens. The structure of the nectaries was analyzed by means of paraffin sections (Li 2003).

Visiting insects

The behavior and frequency of visiting insects during the full blooming stage (about 36 hours) of each species in different spots was recorded (Table 1). Visiting insects were captured for later identification, to observe the part of the insect carrying the pollen and to record the quantity of pollen. Weak, long wavelength light from an emergency lamp with a red shade was used to carry out night observations. All the observations were made on sunny days.

Visiting insects with no obviously visiting purpose were not included. Visiting frequencies (visiting times to an inflorescence per hour) were counted. The parts of the insects carrying the pollen grains were observed under with an anatomical lens and washed in clean water for several times. The liquid obtained was mixed and centrifuged. The mixture in the lower layer was collected and diluted to proper concentration to do the follow counting on hollow-ground slides under a light microscope. The averages number of pollen grains numbers was calculated using 50 individuals of insects with higher visiting frequencies or all individuals with lower visiting frequencies (Moog *et al.* 2002).

Wind pollination

The possibility of anemophily and pollination effectiveness was determined by gravity slide tests. Three slides daubed with Vaseline were placed in four directions at 50cm, 100cm, 200cm, 300cm, 400cm and 500cm distance from two samples of each population. The area of the slide daubed with Vaseline was 2.5 cm × 6.0 cm. After remaining in place for 36 h, the slides were collected to detect the quantity of pollen (Huang *et al.* 1999).

Pollination effectiveness

To determine the effectiveness of pollination, nine pistillate plants of each species at each site were randomly selected and three inflorescences (which were still closed) of each individual were bagged with gauze (mesh size 0.1mm) to test for wind pollination (Goodwillie 1999; Moog *et al.* 2002; de la Bandera and Traveset 2006) and with cellophane to determine the possibility of self-pollination or apomixis. Non-bagged inflorescences were used as a control. Fruit set and seed set were calculated during the fruiting stage (Ma and Huang 2000).

Reproductive mode

Two hundreds seedlings were randomly dugout to determine whether they grew seeds or bulbils.

Seed germination

Thirty seeds collected in the field were immersed in water for 24 h and then transferred to petri dishes in the dark and held at 25°C (Terui and Okagami 1993; Peng *et al.* 2002). Observations were made every 24h. When the radicle was roughly as long as the seed, it was scored as germinated. When the seed did not germinate over 5 consecutive days, it was recorded as not germinating. All observations were done with three repeats.

3. Results

Sex ratio and distribution

According to our observations, 48% of the staminate and pistillate plants of wild *D. zingiberensis*

on Mt. Wudang were less than 1 m apart, and 72% were less than 5 m apart. The pistillate-staminate ratio was 1: 1.56 for 225 flowering plants in which there was an individual with staminate and pistillate flowers on different stems, one with staminate and pistillate flowers in different inflorescences on the same stem and one individual with staminate and pistillate flowers in the same inflorescence. *Dioscorea zingiberensis* in the wild often has only one twining stem from each rhizome, but occasionally there are several stems; the most we observed was 11.

Table 1. Species used for observation of visiting insects

Taxon	Location	Pistillate plant		Staminate plant	
		Number of individuals	Number of inflorescences	Number of individuals	Number of inflorescences
D. zingiberensis	Purple Heaven Palace, Mt. Wudang,	2	49	3	301
	South Rock, Mt. Wudang	2	17	1	114
D. polystachya	Purple Heaven Palace, Mt. Wudang,	4	505	4	992
	Mozi Gully, Yingxiu	2	246	2	739
D. bulbifera	Mozi Gully, Yingxiu	5	48		
	Shaohuoping, Yingxiu			9	105
	Mayidui, Lincang	6	888	6	597

Wild staminate and pistillate plants of *D. polystachya* on Mt. Wudang occur in patches, and the distribution areas are far apart. In our survey, 194 flowering plants were found over an area of 11 km^2, in which the pistillate-staminate ratio was 1: 2.4. Sixteen percent of the staminate and pistillate plants were within 1 m of each other, and 35% were within 5 m. Wild staminate and pistillate plants of *D. polystachya* in Yingxiu were distributed evenly. Three hundred ninety four flowering plants were found over an area of 5 km^2. The pistillate-staminate ratio was also 1: 2.40 as well. Thirty eight percent of staminate and pistillate plants were less than 1 m apart, and 61% were less than 5 m apart.

Wild staminate and pistillate plants of *D. bulbifera* in Yingxiu were distributed discontinuously. The individuals on one side of the Min River were all pistillate, and most of the plants on the other side of the river were staminate, with few pistillate individuals. Staminate and pistillate individuals were at least 300 m apart. The pistillate-staminate ratio was 1.17: 1 for the 104 flowering plants we found. Staminate and pistillate plants of *D. bulbifera* at Mayidui grew intermixed. The pistillate-staminate ratio was 11:13 and the distance between them was 2-10 m.

Floral traits and flowering phenology

The average number of staminate inflorescences of *D. zingiberensis* was 295. Staminate inflorescences (Figure 1a) had 6-31 florets during 10-12 d. Staminate flowers (Figure 1c) are sessile, solitary or in cymules of 2-3 flowers with 3-4 bracts. The petals were purplish red, 1.20 ± 0.37 x 1.10 ± 0.41 mm wide, spreading at anthesis, and opening to 3.0 ± 0.6 mm in diameter. The six stamens were inserted at the margin of the receptacle. and the filaments were extremely short. The flowers had no nectar or aroma. The average number of pollen grains per flower was 1147 ± 76. The size of open staminate flowers was the same both day and night. The average number of pistillate inflorescences was 49. The pistillate inflorescences (Figure 1b) had 1-15 flowers. The blooming period was 8-10 d. Pistillate flowers (Figure 1d) were sessile, solitary and have the same number of petals as the staminate flowers. The staminodes were filiform. The stigma was moist and 3-lobed. No nectar or aroma was detected. The open flowers had the same dimensions during the day and at night.

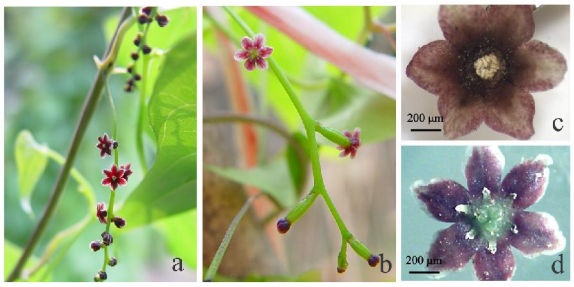

Figure 1. Floral traits of *Dioscorea zingiberensis*: (a) staminate inflorescence; (b) Pistillate inflorescence; (c) staminate flower; (d) Pistillate flower.

The staminate inflorescences of *D. polystachya* (Figure 2a) had 2 - 9 flowers together, rarely paniculate arranged. The staminate flowers (Figure 2c) had two ovate bracts and were 0.34 ± 0.12 mm in diameter. The six perianth lobes were yellow with purplish brown dots. The outer lobes were broadly ovate, 1.76 ± 0.15 ×1.40 ± 0.10 mm. The inner lobes were ovate and smaller than the outer. Six stamens were inserted at the base of the perianth. The staminate flowers opened slightly at 04:30 am, became fully open at 06:00 am, then

started to close at 02:00 pm and were fully closed at 12:00 pm when the fragrance became undetectable. The pistillate inflorescences (Figure 2b) were solitary or 2-6 together. The fragrant pistillate flowers (Figure 2d) had 2 lanceolate bracts, and opened to 0.49 ± 0.09 mm in diameter. The color and shape of the perianth lobes was the same as in the staminate flowers. The stigma was moist. The staminate flowers were open the same amount by day and at night. We noted differences in number of staminate inflorescences, number of flowers and length of inflorescences between the populations of *D. polystachya* on Mt. Wudang and at Yingxiu, i.e. (Table 2).

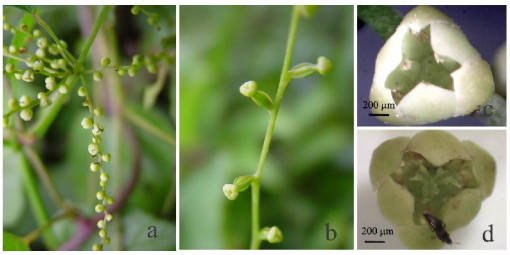

Figure 2. Floral traits of *Dioscorea polystachya*: (a) staminate inflorescence; (b) Pistillate inflorescence; (c) staminate flower; (d) Pistillate flower.

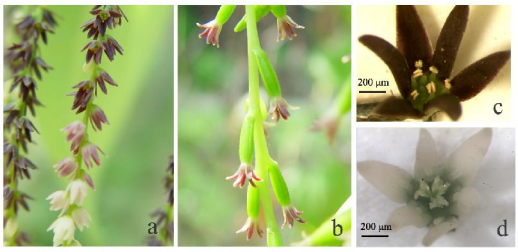

Figure 3. Floral traits of *Dioscorea bulbifera*: (a) staminate inflorescence; (b) Pistillate inflorescence; (c) staminate flower; (d) Pistillate flower.

The average number of staminate inflorescences for *D. bulbifera* was 426. The staminate inflorescences (Figure 3a) are 2-6 together, rarely paniculate, and 23-69 flowered. The staminate flowers (Figure 3c) had 2 ovate bracts. The six tepals opened at a 45-degree angle. They were initially white, and then turned pink to purplish red with age. The outer lobes were lanceolate, 2.15 ± 0.11 × 0.52 ± 0.03 mm; the inner lobes were 2.07 ± 0.10 × 0.83 ± 0.08 mm. Six stamens were inserted at the base of the perianth. The average number of pollen grains per flower was 5420 ± 164. The aroma and openness of the staminate flowers was the same during day and night. The pistillate inflorescences (Figure 3b) have 12 - 46 flowers. The pistillate flowers (Figure 3d) were solitary, with 2 lanceolate bracts, and the tepals were held at a 45-degree angle. Six perianth lobes were white and 1.29 ± 0.14 × 0.40 ± 0.17mm. The stigma was 3-lobed. The flowers had no aroma. The open flowers were the same during the day and at night.

Structure of floral nectary

The pistillate flowers of *D. zingiberensis* have three nectar glands located in three cavities formed by of the carpels with nectariferous tissues around (Figure 4a). The secretory epidermis has a layer of specialized palisade cells arranged in the form of palisade. The nectariferous tissue has comprises 1 -or 2 layers of densely arranged small cells with no starch grains or vascular bundles (Figure 4b). The three nectar glands are separated at the top of the stigma, with the nectar flowing from the opening of the stigma (Figure 4c) and are joined at the bottom, with no opening (Figure 4d). The pistillate flowers of *D. polystachya* and *D. bulbifera* have similar nectaries. The staminate flowers of all three species have no nectary.

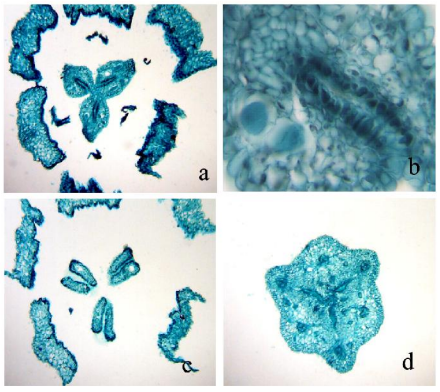

Figure 4. Nectary of *Dioscorea zingiberensis* 10×: (a) floral nectary; (b) nectariferous tissue; (c) opening of nectary; (d) joined nectaries.

Species, behavior, visiting frequency and quantity of pollen carried of visiting insects

The only insect visiting *D. polystachya* on Mt. Wudang and at Yingxiu was *Ernothrips lobatus* (Figure 5a). When the flowers start to open at 4: 30 am, 1 or 2 thrips crawled in from the opening, continued to drift in and out, sometimes rested in the flowers, and sometimes gnawed the anthers. As the number of open flowers increased and fragrance strengthened, more thrips arrived. When a male thrip attempted to enter a flower that was already occupied by another male, there was always a fight until the winner drove the loser out of the flower. If the second visitor was a female thrip, it crawled into the flower, then stayed for a while or left. The peak scramble lasted for 8-9 hours, during which there were thrips

in at least 2/3 of the open flowers, and up to 14 thrips in one flower. After 06:00 pm, visits by insects diminished significantly. The staminate flowers closed at night and no thrips could enter or leave the flowers, but when the inflorescences were shaken gently, thrips fell from the flowers.

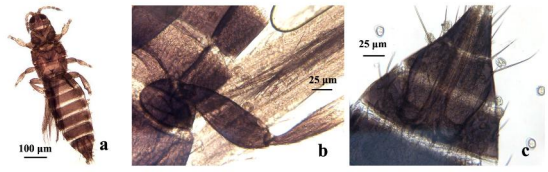

Figure 5. Features of *Ernothrips lobatus*: (a) Adult insect; (b) Appendages with pollen grains; (c) Abdomen with pollen grains

When thrips visited, they usually crawled on the same inflorescence, and did not fly until they left the plant, so the revisitation rate was high. Of concern were the many white thrip larvae we found on the flowers and thrip eggs in floral tissues, indicating a symbiotic relationship between *Ernothrips lobatus* and *D. polystachya.*

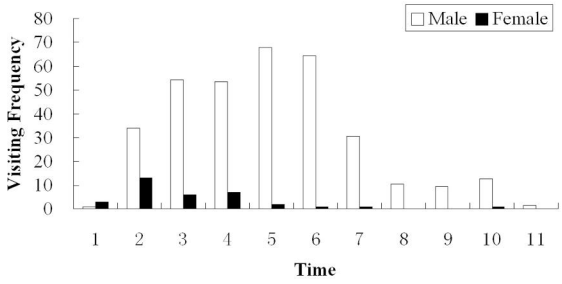

Figure 6. Frequency of insects visiting flowers of *Dioscorea polystachya*

Ernothrips lobatus has sparse hairs. Pollen grains were found on the head, abdomen, notum, appendages and wings (Figure 5b, c). The average number of pollen grains carried by thrips in staminate flowers was about 1/5 of thrips in pistillate flowers which are 1 m away, and the average number is 4.81. Only 40% of thrips in pistillate flowers 5 m away from staminate flowers were carrying pollen grains, and the average number of pollen grains per thrip was only 0.68. There were no pollen grains on thrips in pistillate flowers 10 m away from staminate flowers. The pollination distance of thrips is therefore limited. Data obtained at Mt. Wudang and Yingxiu is similar. Thrips visit staminate flowers more often than pistillate flowers (Figure 6). The peak visiting time to staminate flowers was from 09:00 am to 18:00 pm, while to pistillate flowers it was from 09:00 am to 24:00 am.

At the two study sites of *D. zingiberensis* and *D. bulbifera*, we found no insects visiting in two consecutive years. Occasionally some members of the *Hymenoptera* approached the flowers of *D. bulbifera*, but left before long and showed no pollination behavior.

Possibility of wind pollination

Ninety percent of the pollen of *D. zingiberensis* on Mt. Wudang was dispersed within 2 m of the pollen source. Only 6.25% of the pollen was dispersed to 3 m away, and less than 1% at 5 m distances (Figure 7). *Dioscorea polystachya* does not achieve long-distance pollen dispersal by wind.

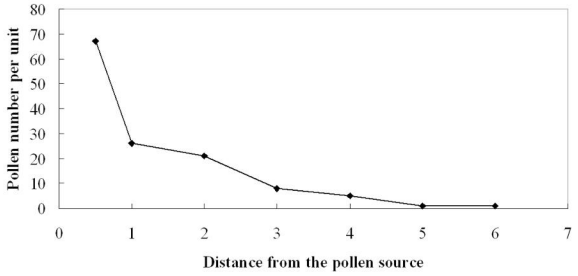

Figure 7. Pollen dispersal by wind of *Dioscorea zingiberensis*

The possibility of wind pollination was ruled out, since no pollen was found on the slides placed around *D. polystachya* at Mt. Wudang and Yingxiu.

Ninety percent of the pollen of *D. bulbifera* at Yingxiu was dispersed within 3 m, and mostly within 2 m, of the pollen source. Less than 2% was 5 m away (Figure 8). The pollen fell to the ground by gravity rather than being dispersed by wind. The shortest distance between staminate and pistillate individuals of *D. bulbifera* at Yingxiu was 300 m, so the main pollination method could not have been wind.

The ratio of white, pink and purple flowers of *D. bulbifera* at Mayidui was 1: 2.82: 3.13. Moreover, 80% of the pollen was dispersed within 2 m of the pollen source.

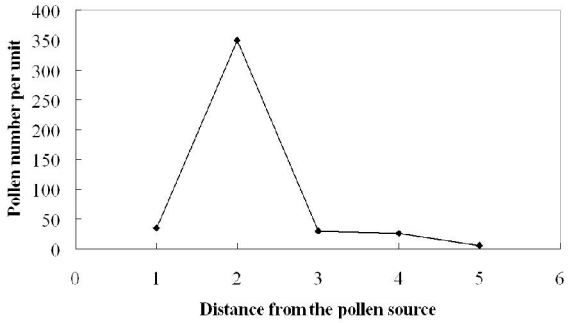

Figure 8. Pollen dispersal by wind of *Dioscorea bulbifera*

Table 2. Floral traits of *Dioscorea polystachya* on Mt. Wudang and at Yingxiu

Characteristics	Staminate flowers			Pistillate flowers		
	Mt. Wudang	Yingxiu	Significance	Mt. Wudang	Yingxiu	Significance
Number of inflorescences in one plant	359.40±307.16	1111.70±994.61	$P<0.05$	104.90±87.23	173.00±87.81	$P>0.5$
Number of florets in one inflorescence	15.87±1.33	21.66±1.26	$P<0.01$	7.08±1.23	10.15±1.10	$P<0.01$
Length of inflorescences	3.26±0.19	4.29±0.23	$P<0.01$	6.23±1.01	8.74±0.84	$P<0.01$

Pollination effectiveness

Under natural conditions, fruit set in *D. zingiberensis* in Mt. Wudang was 50.71% and seed set was 32.89%. The cellophane bagged group had no fruits or seeds. Fruit and seed set in plants bagged in gauze was 28.57% and 11.11%, respectively. No visiting insects were observed and there was no self-pollination, so it can be concluded that *D. zingiberensis* is wind pollinated. In addition, fruit and seed set in the gauze bagged group were lower than in the control group, suggesting that the gauze bag hinders pollination by wind.

Fruit set and seed set under natural conditions in *D. polystachya* on Mt. Wudang was 68.52% and 41.87%, and in *D. polystachya* in Yingxiu, 62.42% and 48.74 %. Gauze bags with a mesh size of 0.05 mm completely prevented thrips from visiting the flowers and reduced fruit and seed set to zero. When bagged with 0.20 mm gauze, the fruit sett and seed set of *D. polystachya* on Mt. Wudang was 24.35% and 13.33%, and zero in *D. polystachya* in Yingxiu. The results under natural conditions indicate that when thrips are kept out, fruit and seed set in *D. polystachya* is influenced seriously, but as long as a small number of thrips visit the flowers, the plants are pollinated. No fruit was in the cellophane bagged group, indicating the absence of apomixis.

Fruit and seed set under natural conditions in *D. bulbifera* in Yingxiu was zero, and 56.5% and 90.3% in the Mayidui populations. No fruit was in the cellophane bagged group, indicating no apomixis.

Reproductive mode

Dioscorea zingiberensis does not produce bulbils, so all the seedlings on Mt. Wudang were derived from seeds. Two hundred and fifty three seedlings of *D. polystachya* were found on Mt. Wudang, of which 225 were from bulbils, accounting for 88.93%. Twenty eight seedlings were from seeds, accounting for 11.07%. Although insect pollination occurs in this region, the dominant reproductive mode is asexual. Two hundred and fifty seedlings of *D. polystachya* were observed at Yingxiu, of which 13 were from bulbils, accounting for 5.20%. The 237 seedlings from seeds accounted for 94.80%. Of 394 plants observed, only 5 had bulbils.

Two hundreds seedlings of *D. bulbifera* observed at Yingxiu were from bulbils. There was no evidence of insect or wind pollination, so the dominant reproductive mode must have been asexual. Of the 144 (86.11 %,) seedlings of *D. bulbifera* observed at Mayidui, 124 were from bulbils. The 20 derived from seeds accounted for 13.89%. In this region, reproductive is mainly asexual, with wind pollination being an alternative.

Seed germination

Seeds of *D. zingiberensis* on Mt. Wudang began to germinate after 7 d, with a duration of about 20 d. The average germination rate for two consecutive years was 71.4%. Seeds of *D. polystachya* in Yingxiu started to germinate after 7 d, with the duration being about 20 d. The average germination rate over two consecutive years was only 8-9%.

D. polystachya from Mt. Wudang and *D. bulbifera* in Sichuan and Yunnan propagate vegetatively, so their germination was ignored.

4. Discussions

Relationship between floral traits and pollination

Burkill (1960) predicted that all species of Dioscoreaceae are insect pollinated and there is no possibility for wind pollination. Our research determined otherwise.

Dioscorea zingiberensis and *D. nipponica* subsp. *rosthornii* belong to the same section *Stenophora*. The latter has a large number of yellowish green inflorescences and fragrant flowers with nectar, which are typical traits of insect pollinated flowers. In contrast, the flowers of *D. zingiberensis* are aubergine with no aroma and no nectar. The anthers face outward, the perianth is flat, and the flowers are sparsely distributed. Judging from the morphology, *D. zingiberensis* is much more likely to be wind pollinated. During two-year years of observation, we found no insects visiting *D. zingiberensis*. Pollen grains were dispersed within 5 m of the staminate plant. Moreover, since *D. zingiberensis* produces only a single stem each year (Wang *et al.* 2010), it may have an alternative method of reproduction. The limited number of sticky pollen grains are inimical to wind pollination.

In fact, 90% of the pollen grains are dispersed within 2 m of their source, but the mixed distribution of staminate and pistillate plants in the wild made up for that in large extent. Wind pollination is therefore the only likely means of effective pollination in *D. zingiberensis*. *Dioscorea zingiberensis* is the most primitive species in sect. *Stenophora*. It is endemic to China and is currently widely distributed from eastern to western China, including Henan, Hubei, Shanxi, Gansu, Sichuan and other regions. *Dioscorea zingiberensis* and *D. nipponica* are closely related and occur in overlapping geographic areas, but employ completely different pollination methods, indicating a divergence during the evolution of *Dioscorea* species and implying primitiveness and diversity in sect. *Stenophora*.

Terry (2002) concluded that flowers pollinated by thrips should be small, globose, and tight with an opening of 0.3 - 0.5 mm, usually light green or yellow, fragrant. The staminate and pistillate flowers of *D. polystachya* satisfy these qualifications. Our study in Hubei and Sichuan proved that *Ernothrips lobatus* is the pollinator and *D. polystachya* provides thrips with living and breeding sites. The number of inflorescences and flowers, as well as inflorescence length of *D. polystachya* in Yingxiu, Sichuan is significantly greater than in plants on Mt. Wudang, but they have a similar degree of fruit and seed set, indicating that the pollination ability of thrips is not affected by floral traits.

The flowers of *D. bulbifera* remain half closed. The staminate flowers are brightly colored and produce a strong aroma while the pistillate flowers have a nectary. Judging from such floral characteristics, *D. bulbifera* may be insect pollinated, while, no insect were seen in any of our study sites during our observations. This phenomenon may be attributable to the large amount of rainfall in the area (annually 1 253 mm) which makes pollen too wet to disperse. Ninety percent of the pollen of *D. bulbifera* in Yingxiu is dispersed within 2 m from its source, and fruit and seed set was zero, indicating the unlikelihood of wind pollination. All the young plants there were from bulbils, resulting in the disjunct distribution of staminate and pistillate plants. In Mayidui, wild staminate and pistillate plants of *D. bulbifera* grow intermixed, they also do not reproduce through insect pollination. This phenomenon might derive from the fact that among all the white, pink and purple flowers of an inflorescence, purple flowers which, like *D. zingiberensis* flowers, have non-anthophilous features, account for the largest proportion. The fact that pollen grains are dispersed within 2 m of the pollen source and fruit and seed set was respectively 56.5% and 90.3% indicate that *D. bulbifera* is wind pollinated.

The flowers and pollinators adapt to the characteristics of each other. It is generally acknowledged that variation in the corolla is the result of a gradual process. There must be transitional types (Wang *et al.* 2003). The staminate flowers of sections *Lasiophyton*, *Botryosicyos* and *Enantiophyllum*, which produce bulbils are small and nearly closed, while staminate flowers of sect. *Opsophyton* are half closed. The staminate flowers in sect. *Stenophora*, *Combilium*, *Stenocorea* and *Shannicorea* are larger and open widely. According to the theory that asexual reproduction is more evolved than sexual reproduction, the evolutionary trend in the flowers of *Dioscorea* ranges from totally open to half closed to nearly closed.

Reproductive strategy and evolutionary significance of the three *Dioscorea* species

Dioscoreaceae are a group of relatively primitive monocots. The species exhibit both asexual and sexual reproduction. Some species reproduce by seeds and rhizomes or tubers, such as those in sections *Stenophora*, *Combilium*, *Stenocorea* and *Shannicorea*. Some species propagate by seeds, bulbils and tubers, such as those in sections *Opsophyton*, *Lasiophyton*, *Botryosicyos* and *Enantiophyllum* (Pei and Ting 1985; Ting and Michael 2000) which all grow from bulbils produced above ground. Under natural conditions, only one twining stem grows from a perennial rhizome or tuber, but occasionally there are two or more. The number of new individuals produced in this way is limited. It is therefore sexual reproduction and asexual reproduction by bulbils that allow the species to increase in number of individuals.

Although the treatment in the *Flora of China* (Ting and Michael 2000) reports *D. zingiberensis* to be dioecious, plants with staminate and pistillate flowers on different stems, plants with staminate and pistillate flowers in different inflorescences on the same stem and plants with staminate and pistillate flowers in the same inflorescence were found in cultivated *D. zingiberensis* (Cao *et al.* 2004; Ce *et al.* 2005), and their sexual expression was reversible (Wang *et al.* 2010). Nevertheless, it is rare for wild individuals to demonstrate such characteristics, indicating that *D. zingiberensis* is at an evolutionary middle stage between hermaphroditism and dioecy. Plants of sect. *Stenophora* have open flowers. Most of them demonstrate typical features of insect pollination. *Dioscorea zingiberensis* has flowers with low wind pollination efficiency. The fact that wind pollination is only possible between plants that are close to each

other requires a relatively high density of distribution. *Dioscorea zingiberensis* in the wild therefore reproduces by developing aerial stems. Even though one stem arises each year, this mode of reproduction maintains a stable number of individuals. According to some studies, the diversity index within populations of *D. zingiberensis* is about 0.2, and gene flow between populations is 0.9641, indicating little genetic exchange. Moreover, the germination rate of seeds of *D. zingiberensis* under natural conditions is also very low (Huang *et al.* 2003). There should therefore be barriers that obstruct sexual reproduction of *D. zingiberensis.*

Most plants of *D. polystachya* on Mt. Wudang reproduce asexually by bulbils while most plants of *D. polystachya* in Yingxiu reproduce sexually by seeds. Why so individuals of the same species in different regions reproduce by completely different modes? *Dioscorea polystachya* is densely distributed in Yingxiu, and staminate and pistillate plants are evenly mixed. Thrips can pollinate the closely spaced plants, so compared with asexual reproduction, sexual reproduction is the major reproductive mode. Plants of *D. polystachya* on Mt. Wudang are sparsely distributed and thrip pollination, which is only possible at close range, is less likely. The main means of reproduction in this population is therefore by bulbils. These plants eventual expand their territory and reduce the distance between staminate and pistillate individuals so that sexual reproduction will eventually be possible. Almost all the bulbils we found at the study sites produced new plants, but the germination rate of seeds was low. Certain obstacles which work against sexual reproduction in *D. polystachya* and insect pollinators are a plausible explanation for the low germination rate.

Dioscorea bulbifera is densely distributed along rivers and in forested areas below 1000 m Yingxiu. Within this group, staminate and pistillate plants have a unique distribution pattern and plants produce flowers. Like *D. bulbifera* at Mayidui, however, no insects were observed to visit the flowers and 80% of the pollen grains were dispersed within 2 m of the pollen source, while only 2% could be detected 5 m from the source. It appears that pollen cannot be dispersed by wind over long distances. Regardless of whether staminate and pistillate plants are distributed in mixed populations or separately, there is neither insect nor wind pollination in *D. bulbifera*. Since sexual reproduction is obstructed, bulbils and tubers have become the main mode of reproduction. The fact that 100% of the young plants in Yingxiu and 86.11% in Mayidui grow from bulbils provides evidence to that effect. In addition, 13.89% of the young plants of *D. bulbifera* in Mayidui are from seeds, indicating that a small number of plants are derived from wind pollination. No such phenomenon, however, was observed at Yingxiu. We deduced that the high humidity in the area prevents pollen from being dispersed, thereby reducing the probability of wind pollination to zero.

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