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RESEARCH PAPER

The comparative reproductive biology of a tetraploid species, *Hedychium villosum*, and its diploid progenitor *H. tenuiflorum* (Zingiberaceae)

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ABSTRACT

The evolutionary advantages of polyploidy may result from a number of changes in floral traits and breeding system, which may enable polyploids to exploit new habitats and become widespread. In this study, we comparatively investigated the floral biology of the tetraploid species *Hedychium villosum* and its diploid progenitor *H. tenuiflorum*, to assess reproductive divergence between the two species. The results showed that flowers of the tetraploid species last longer and produce more nectar than did diploid species. The flowering times of the two species did not overlap at all. Observations of floral visitors in natural populations demonstrated that butterflies and hawkmoths were effective pollinators of both species, but there was a significant difference in butterfly and hawkmoth assemblages between the two species. The hand-pollination experiments and pollen tube growth experiments suggested that diploid *H. tenuiflorum* was self-incompatible, while tetraploid *H. villosum* was completely self-compatible. *H. villosum* has a much wider distribution range and occupies more diverse habitats than *H. tenuiflorum*. Polyploidisation may enable tetraploid *H. villosum* to exploit new habitats previously unavailable to diploid *H. tenuiflorum*.

INTRODUCTION

Polyploidy is a very common feature of flowering plants. Polyploidy plays a very important role in the creation and maintenance of plant biodiversity, and has had a profound influence on the evolutionary history of extant lineages (*e.g.* Leitch & Bennett 1997; Soltis *et al.* 2003, 2009). Recent studies indicate that all angiosperms have had a whole genome duplication during their history (Masterson 1994; Otto & Whitton 2000; Cui *et al.* 2006). Polyploid clades are thought to be different from their diploid progenitors in important traits such as breeding systems, ecological tolerances, growth rates, pollinators and herbivores and rates of adaptation (Campbell *et al.* 1991; Thompson *et al.* 1997; Segraves & Thompson 1999; Miller & Venable 2000; Otto & Whitton 2000; Mable 2004; Thompson & Merg 2008).

Such changes that occurred in many polyploid taxa may influence genetic variation, life histories, physiology and geographic distribution, and lead to the success of those polyploid taxa in nature (Segraves & Thompson 1999; Soltis *et al.* 2009). In general, polyploids occur across a wider range of environments than one of their diploid parents, and are able to exploit habitats previously unavailable to their diploid progenitors (Levin 1983; Barrett & Richardson 1986; Lumaret *et al.* 1987; Husband & Schemske 1998; Soltis *et al.* 2009). In flowering plants, changes in floral morphology and timing of flowering are recognised as two common effects of polyploidy, and are documented in a number of plant species (*e.g.* Tothill & Hacker 1976; Garbutt & Bazzaz 1983; Lumaret *et al.* 1987; MacDonald *et al.* 1988; Lumaret & Barrientos 1990; Brochmann 1993; Maceira *et al.* 1993; Bretagnolle & Lumaret 1995; Petit *et al.* 1997; Segraves & Thompson 1999). Because of these changes, it is not unexpected that changes in pollinator assemblage may follow. However, until recently there have been relatively few studies confirming that plant polyploidy can have profound effects on interactions with pollinators (Segraves & Thompson 1999; Husband 2000; Thompson *et al.* 2004; Thompson & Merg 2008). More cases studies on the reproductive ecology of polyploids and their diploid progenitors are certainly needed in order to better understand the success of polyploids in nature.

The Zingiberaceae is a large clade of animal-pollinated monocotyledons with more than 2000 species in about 50 genera. The family is widely distributed in the tropics, with the highest diversity and number of genera and species in the Asian tropics. *Hedychium* J. König, with more than 50 species, is a large genus widely distributed, from tropical Malaysia to the Himalayan highlands (Larsen *et al.* 1998). Cytological data show that polyploidy is a common feature in *Hedychium*, with the basic chromosome number $n = 17$, and that $2n = 24$, 34, 36, 51, 52, 54, 66 and 68 have also been reported for the genus (Sharma & Bhattacharyya 1959; Mahanty 1970; Mukherjee 1970). There are relatively few studies on the reproductive ecology of *Hedychium* except for very early observations of hawkmoths and butterflies pollinating *H. coronarium* and other species of the Asiatic genus *Hedychium* in Brazil (Müller 1890; Künckel d'Herculais 1910).

Two varieties of *Hedychium villosum*, var. *villosum* and var. *tenuiflorum*, were previously considered to be only morphologically and taxonomically different in plant and flower size (Wu & Larsen 2000), and are sister taxa in a phylogenetic tree (Wood *et al.* 2000; Gao *et al.* 2008). However, a recent study showed that var. *tenuiflorum* (2n = 34) is diploid and var. *villosum* (2n = 68) is tetraploid. The two varieties were also recognised as distinct species because of discernible morphological characters, distinct geographic ranges and habitats, and complete reproductive isolation. The tetraploid var. *villosum* was kept as *H. villosum* and the diploid var. *tenuiflorum* was renewed to *H. tenuiflorum* (Yu *et al.* 2010).

To better understand the success of polyploid taxa in nature, and to assess the influence of polyploidisation on reproductive divergence, we compared the floral biology and breeding systems of these two closely related species. Our study addressed three main questions: (i) what is the differentiation in reproductive traits of the two species; (ii) what are the compatibility systems of the two species; and (iii) is there differentiation in assemblage of insect visitors to the two species?

MATERIAL AND METHODS

Study species and study sites

Both *Hedychium villosum* (tetrapolid) and *H. tenuiflorum* (diploid) are epiphytic small gingers with robust fleshy rhizomes. The spike with many cincinnus is terminal on an erect pseudostem. The cincinnus, a type of monochasium on which the successive axes arise alternately with respect to the preceding one, contains two to three flowers; flowers in the cincinnus open in turn (Wu & Larsen 2000). Although the flowers of both species do not differ in floral shape, each can easily be distinguished morphologically because shoots, leaves, inflorescence and flowers of *H. villosum* are consistently larger than those of *H. tenuiflorum* (Yu *et al.* 2010). The geographic range and habitat of each species is also distinct. *H. villosum* is distributed widely in South China, growing on tall trees or understorey rocks at altitudes of 1500–1800 m. *H. tenuiflorum* is very common only in a narrow area of Xishuangbanna, South China, growing on calcareous rocks on the peaks of limestone mountains at altitudes of 800–1350 m. No sympatric populations of the two species or mixed-ploidy populations have been found (Yu *et al.* 2010).

The study site of *H. villosum* was at a tropical montane forest in Mengsong, Jinghong County (21°27′ N, 100°25′ E; 1500 m a.s.l.). The forest is dominated by *Parachmeria yunnanensis*, *Gymnanthes remota*, *Mastixia euonymoides* and *Phoeba megacalyx* (Zhu *et al.* 2004). Plants of *H. villosum* grow on the top branch of tall trees (Fig. 1a). Investigations on *H. tenuiflorum* were conducted at Qingshizhai, Mengla County (21°48′ N, 101°23′ E; 1085 m a.s.l.). The study site was a limestone monsoon rain forest mountain with sparse trees and shrubs, including *Photinia anguta* var. *hookeri*, *Pistacia weinmannifolia*, *Myrsine semiserrata* and *Pterospermum proteum* (Zhu *et al.* 2003). Plants of *H. tenuiflorum* grow on calcareous rocks of mountaintops (Fig. 1b).

In addition, observations of flowering phenology and some hand-pollination experiments were carried out in the Wild Ginger Collection of Xishuangbanna Tropical Botanical Garden (XTBG). The main study period was during the flowering and fruiting seasons of the two species from 2004 to 2007.

Reproductive traits

Preliminary phenology of both species was monitored aperiodically in the study sites from 2004 to 2007. Detailed observations on flowering phenology were made by recording the number of opening flowers per inflorescence per day, the time of flower anthesis and withering, and anther dehiscence during the flowering period.

To study nectar secretion, 30 inflorescences of each species were randomly selected and bagged before anthesis. We used 10-μl Sigma 'micro-cap' calibrated capillary tubes (Sigma Chemical Co., St. Louis, MI, USA) to measure the nectar volumes. Each of 30 flowers of first-day, second-day and third-day flowers were randomly selected and measured from 11:00–13:00 h on 9 March 2005 for *H. villosum*, and on 19 September 2006 for *H. tenuiflorum*. Nectar sucrose concentration of each flower was measured with a hand-held, temperature-compensated refractometer (Eclipse; Bellingham & Stanley Ltd., Basingstoke, Hampshire, UK) at the same time.

Hand-pollination experiments

To determine self-compatibility, we carried out different pollination treatments on the two species. For *H. villosum*, four treatments were conducted on 107 inflorescences of 46 individuals in the study site during 22–28 February 2005 as follows: (i) bagging: 30 inflorescences of 14 individuals were bagged throughout without pollination; (ii) selfing: 24 inflorescences of 12 individuals were hand-pollinated with pollen from the same flower; (iii) crossing: 23 inflorescences of ten individuals were hand-pollinated with the pollen of another individual; and (iv) control: 30 inflorescences of 30 individuals were marked for natural pollination. The fruit set of each treatment was counted about 4 weeks later (on 2 April). For *H. tenuiflorum*, the same four treatments were made on 15 inflorescences of 15 individuals for each treatment in the study site during 1–16 October 2004, and the fruit set of each treatment was counted about 8 weeks later (on 6 December).

For the selfing and crossing treatments, inflorescences were bagged with nylon mesh before anthesis and bagged again after hand-pollination. In all treatments, the number of open flowers was recorded. The seed number per fruit was counted in the laboratory from 60 randomly selected fruits for each treatment. We used the mean ovule number of each species to count the seed set (seed:ovule ratio).

Pollen tube growth experiments were also carried out to compare the compatibility of self- and cross-pollination. We bagged inflorescences on the day before flowers opened and performed selfing and crossing hand-pollination treatments on each species. At 2, 4, 8, 12, 16, 24, 36 and 48 h after pollination, we picked 12 flowers per treatment, removed the styles and immediately fixed them with FAA solution (formaldehyde, acetic acid, 70% ethanol at 5:5:90, v/v/v), transferred them to ethanol (70%) after 24 h of fixation, and then stored them in a refrigerator at 5–8 °C. Pollen tubes were measured following the aniline blue method as described in Dafni (1992). The growth rate of pollen tubes (v) was determined as the length of pollen tube (Lpt) divided by the total style length (Ls), *i.e.* v = Lpt/Ls.

Fig. 1. Habitats and visitors of the diploid *Hedychium tenuiflorum* and the tetraploid *H. villosum*. a: *H. villosum* usually grows on the top branches of tall trees. b: *H. tenuiflorum* usually grows on rocks. c: A butterfly visiting the inflorescence of *H. villosum*. d: A butterfly, *Hyarotis adrastus prabus*, visiting the inflorescence of *H. tenuiflorum*. e: A hawkmoth, *Macroglossum stellatarum*, visiting the inflorescence of *H. villosum*. f: A sunbird visiting the inflorescence of *H. villosum*. g: A fly was seen frequently visiting *H. tenuiflorum*, and climbing on the style searching for pollen grains. h: A nectar-robbing bee-fly was seen frequently visiting flowers of *H. villosum*.

Floral visitor observations and experiments on pollinator efficiency

We discontinuously observed animal visitors to *H. villosum* at the study site between 08:00 and 21:00 h in 2005 (5–9 March) and 2006 (23–27 February), for a total of about 86 h. For *H. tenuiflorum*, observations were conducted from 08:00 to 19:30 h continuously in 2004 (11–15 November) and 2005 (3–8 October) at the study site, with a total observation time of about 106 h. The duration and frequency of visits as well as the behaviour of the pollinators were recorded. All visitor species were photographed and captured for identification and for measurement of body and wing size after observations were completed.

The following three experiments were carried out to compare pollination efficiency of nocturnal and diurnal visitors to both species: (i) bagging diurnally: inflorescences were bagged from 08:00 to 18:00 h; (ii) bagging nocturnally: inflorescences were bagged from 18:00 to 08:00 h; and (iii) control: inflorescences were unbagged all day. For *H. tenuiflorum*, the experiments were conducted on 16 individuals from 30 September to 6 October 2005. Three inflorescences were selected from each of the 16 individuals for the three experiments. Five weeks after experiments were completed, fruit set was counted on 11 November. For *H. villosum*, three inflorescences were also selected from each of the 15 individuals for the three experiments. The experiments were run from 25 February to 6 March 2006, and fruit set was counted on 21 April.

Statistical analysis

All statistical analyses were performed using the statistical program SPSS (version 13.0; SPSS Inc., Chicago, IL, USA). Nectar

volume was compared on species and flower opening times with a two-way ANOVA. The data on fruit set of different hand-pollination treatments and the control experiments were arcsine transformed, then compared with one-way ANOVA, and Tukey's honestly significant difference test was used to analyse the variances among different treatments, in which the differences among fruit sets of different hand-pollination treatments were determined, and the pollination efficiency between diurnal and nocturnal visitors compared for each species. The seed set ratios of different hand-pollination treatments were compared using a one-way ANOVA or with a *t*-test, where only two data points were available.

RESULTS

Reproductive traits

Our observational results, from 2004 to 2007, indicate that the flowering time of the diploid species, *H. tenuiflorum*, and the tetraploid species, *H. villosum*, do not overlap at all. Flowering occurs mainly from late September to mid-November in *H. tenuiflorum* and from late February to early April in *H. villosum*, but the timing of anthesis of the two species is almost the same. Flowers fully open at dusk (18:00–20:00 h), and anthers dehisce and begin to release pollen grains at the same time. Like other species in the genus, the anther consists of two adjacent anther sacs. Pollen grains are covered with a glue-like substance secreted by hairs on the side of each anther sac. The floral longevity of *H. villosum* is 5 days and *H. tenuiflorum* is 4 days.

Flowers of *H. villosum* secreted significantly more nectar than those of *H. tenuiflorum*, and nectar volume increased significantly following the increase of flower opening time for both species (two-way ANOVA on nectar volume, $F_{5,\ 174} = 51.63$, $P < 0.001$; species, $F_{1,\ 174} = 74.84$, $P < 0.001$; flower opening time, $F_{2,\ 174} = 83.02$, $P < 0.001$; and species × flower opening time interaction, $F_{2,\ 174} = 8.62$, $P < 0.001$). However, the nectar sucrose concentrations of flowers at different opening stages were stable for both species (Table 1).

Hand-pollination experiments

Overall, there are significant differences in fruit set among different hand-pollination treatments and the control experiments for both *H. villosum* ($F_{6,\ 173} = 100.72$, $P < 0.001$) and *H. tenuiflorum* ($F_{5,\ 206} = 143.24$, $P < 0.001$, one-way ANOVA on arcsine transformed fruit set).

Table 1. Nectar volume and sucrose concentration of flowers at different opening times in the diploid *Hedychium tenuiflorum* and the tetraploid *H. villosum* (mean ± SD, n = 30).

	H. tenuiflorum		*H. villosum*	
	nectar volume (μl)	concentration (%)	nectar volume (μl)	concentration (%)
first-day flowers	1.83 ± 0.16	24.48 ± 0.40	3.06 ± 0.16	24.70 ± 0.09
second-day flowers	3.75 ± 0.25	24.47 ± 0.36	6.12 ± 0.32	24.43 ± 0.14
third-day flowers	5.09 ± 0.55	23.72 ± 0.40	9.37 ± 0.55	24.83 ± 0.37

In pollination treatments of *H. tenuiflorum* in 2004, we did not find any fruits in the bagging and selfing treatments, and fruit-set in the crossing treatment (81.80% ± 4.97% n = 15 inflorescences) was significantly higher than in the control treatment (8.59% ± 1.38%, n = 87; $P < 0.001$). In pollination treatments of *H. villosum* in 2005, six fruits were found in the bagging treatment (n = 12 inflorescences containing 980 flowers in total). Fruit sets did not differ significantly between the selfing (95.48% ± 9.30%, n = 24 inflorescence) and crossing treatments (91.58% ± 1.51%, n = 23; $P = 0.974$), but both treatments had significantly higher fruit sets than the control treatment (18.59% ± 2.29%, n = 107; $P < 0.001$; Fig. 2).

Analysis of seed:ovule ratio indicated that natural seed sets (50.95% ± 2.76%, n = 60) were significantly higher than crossing seed sets (42.79% ± 3.29%, n = 60) for *H. tenuiflorum* (*t*-test on arcsine transformed seed set, $P = 0.038 < 0.05$). For *H. villosum*, the seed sets of the two hand-pollinated treatments were significantly higher than the control treatment, and selfing was significantly higher than crossing (one-way ANOVA on arcsine transformed seed-set, $F_{2,\ 177} = 101.13$, $P < 0.001$). The mean percentage seed sets for the selfing, crossing and control treatments were 35.53% ± 1.87% (n = 60), 30.20% ± 1.18% (n = 60) and 8.07% ± 1.04% (n = 60), respectively (Fig. 2).

The pollen tube growth experiments showed that pollen germination and pollen tube growth for selfing and crossing treatments did not differ for either *H. tenuiflorum* or *H. villosum* (Fig. 3). Pollen grains started to germinate 4–8 h after hand-pollination and the pollen tube reached 60% of the style length after 48 h for *H. villosum*. Pollen germination and pollen tube growth of *H. tenuiflorum* were faster than for *H. villosum*. Pollen grains started to germinate 4 h after hand-pollination and the pollen tube reached the ovary after 48 h in *H. tenuiflorum* (Fig. 3).

Floral visitor observations and experiments on pollinator efficiency

In total 106 h of visitor observations were made in 2004 and 2005. Three insect species were found visiting flowers of *H. tenuiflorum*. One species of butterfly (*Hyarotis adrastus prabus*) usually visited flowers of *H. tenuiflorum* between 10:00 and 15:00 h on sunny days. *H. adrastus prabus* individuals visited many flowers of up to six inflorescences during one visit. The mean wing length of *H. adrastus prabus* was 20.50 mm (n = 7), which allowed for easy contact with the stigmas and anthers of *H. tenuiflorum* when it rested on the labellum to suck nectar (Fig. 1d). Another effective pollinator of *H. tenuiflorum* was a hawkmoth (*Sphecodina caudata*), which appeared at dusk or overcast days, and had a wing length of about 22.67 mm (n = 3). A small fly also visited flowers of *H. tenuiflorum* frequently and crept on to the filament, lapping pollen grains (Fig. 1g).

In contrast to *H. tenuiflorum*, many animals were observed visiting flowers of *H. villosum*. A sunbird was observed visiting inflorescence on one occasion, but it stayed a very short time and did not touch any parts of the floral organs (Fig. 1f). Beeflies (Bombyliidae) visited flowers of *H. villosum* very frequently, but we regard them as nectar robbers because we never observed contact with stigmas or anthers (Fig. 1h). Both

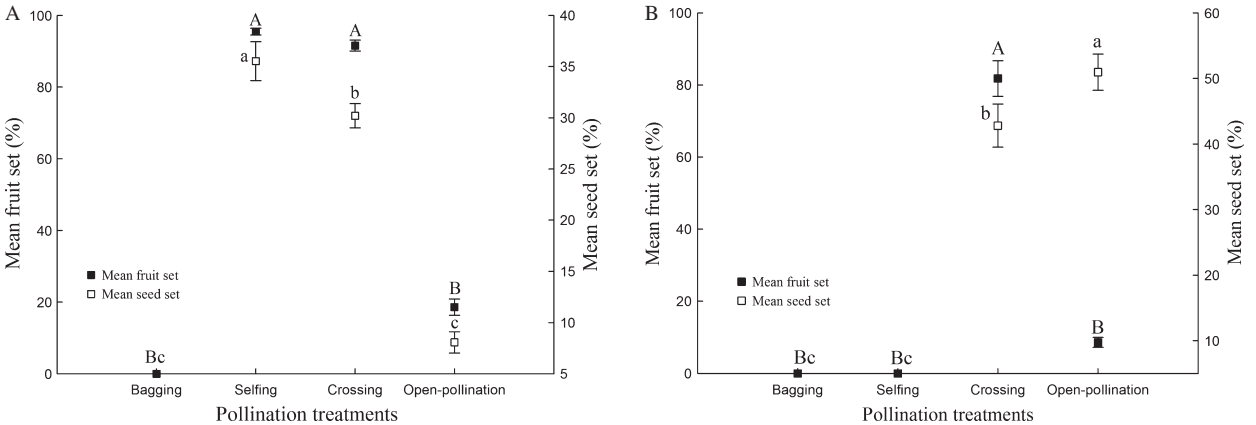

Fig. 2. The effects of pollination treatments on the fruit set and seed set of *Hedychium villosum* (A) and *H. tenuiflorum* (B). Plotted are the means ± SE of the bagging, selfing, crossing and open-pollination treatments. Statistically homogeneous groupings based on a one-way ANOVA and Tukey's HSD test are indicated by the same letter (A or B for fruit set and a, b or c for seed set) above the bars.

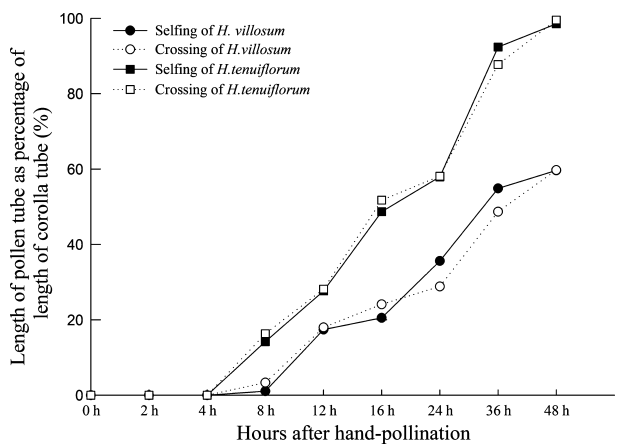

Fig. 3. The pattern of pollen tube growth in the style under two pollination treatments of diploid *Hedychium tenuiflorum* and tetraploid *H. villosum*.

butterflies and hawkmoths were effective pollinators of *H. villosum*. The hawkmoth, *Macroglossum stellatarum* visited flowers of *H. villosum* very regularly from 19:30 to 20:00 h. During this time span, a mass of individuals hovered around the inflorescences of *H. villosum*, and visited flowers one-by-one (Fig. 1e). *Macroglossum stellatarum*, with mean wing length 33.16 mm (n = 4), was bigger than the hawkmoth *Sphecodina caudata* that we observed visiting *H. tenuiflorum*. Butterflies visited flowers of *H. villosum* diurnally (Fig. 1c). Twelve butterflies from seven species were identified: *Isoteinon lamprospilus*, *Mycalesis sangaca*, *Aeromachus stigmatus shanda*, *Polytremis discreta*, *Gerosis phisara tenebrosa*, *Matapa cresta* and *Sarangesa dasahara*. All were similar in appearance, with mean wing length about 19.73 mm (n = 12).

The total fruit set of the control treatment (48.46% ± 4.02%, n = 15) was significantly higher than the diurnal bagging treatment (27.16% ± 5.47%, n = 15) and the nocturnal bagging treatment (30.35% ± 4.54%, n = 15) for *H. villosum* ($P < 0.001$), but the fruit set did not differ significantly between the diurnal and nocturnal bagging treatments ($P = 0.12$). The fruit set of *H. tenuiflorum* for the diurnal bagging, nocturnal bagging and control treatments did not differ significantly (diurnal bagging *versus* nocturnal bagging 2.58% ± 1.11% *versus* 3.17% ± 1.62%, $P = 1.000$; diurnal bagging *versus* control treatments 7.79% ± 2.34%, $P = 0.999$; and nocturnal bagging *versus* control treatments, $P = 1.000$, respectively).

DISCUSSION

Divergence in reproductive traits and pollinators

In flowering plants, changes in floral morphology and timing of flowering are recognised as two common effects of polyploidy (Segraves & Thompson 1999). As expected, such differences were also apparent in *H. tenuiflorum* and *H. villosum*. The plants, leaves and flowers of the tetraploid species, *H. villosum*, are consistently larger than those of the diploid species, *H. tenuiflorum*, and *H. villosum* has more cincinnus, pollen grains and ovules per unit than *H. tenuiflorum* (Yu *et al.* 2010). Moreover, the flowers of *H. villosum* produced more nectar than those of *H. tenuiflorum* (Table 1). Our results are consistent with previous morphological studies in ploypoids (*e.g.* MacDonald *et al.* 1988; Chmielewski 1994; Bretagnolle & Lumaret 1995; Segraves & Thompson 1999). The so-called 'gigas' effect refers to the increased cell size associated with polyploidy in plants, often resulting in increased organ size throughout the plant (Stebbins 1971). Although we have not made a quantitative assessment of cell size, the changes in floral morphology between the two species might be attributed to the gigas effect.

Differentiation in the flowering times of diploid and polyploid taxa is common, especially when there is little spatial separation between diploid and polyploid populations (Lumaret & Barrientos 1990; Petit *et al.* 1997; Segraves & Thompson 1999). Many of the polyploid species that exhibit differences in flowering time are wind-pollinated, implying that changes in other floral traits would not be as effective in preventing inter-cytotype crosses (Segraves & Thompson 1999). Ginger plants are mainly animal pollinated (Larsen *et al.* 1998). Here, the flowering times of the two study species are completely different. Following the change in flowering time, changes in pollinator assemblage also occurred.

Flowers of the tetraploid species, *H. villosum*, and the diploid species, *H. tenuiflorum*, do not differ obviously in shape, and

show many characteristics of typical butterfly or hawkmoth pollination syndromes, such as long and narrow floral tubes, abundant nectar, fragrance, pale colour, filament extending anther and stigma far from corolla. Our observations demonstrated that butterflies and hawkmoths were both effective pollinators of these two species. Butterflies were diurnal visitors and hawkmoths were nocturnal visitors. The results of control experiments indicated that butterflies and hawkmoths had the same pollinating efficiency and contributed equally to fruit set for both species. However, there was a marked difference in butterfly and hawkmoth assemblages between the diploid species and the tetraploid species. Only one butterfly, *Hyarotis adrastus prabus*, and one hawkmoth, *Sphecodina caudata*, were observed visiting flowers of the diploid *H. tenuiflorum*, compared with seven identified butterfly species and one hawkmoth (*Macroglossum stellatarum*) visiting flowers of the tetraploid *H. villosum*. Moreover, corresponding with the larger flower size and nectar volume in the tetraploid species, *M. stellatarum* is larger than *S. caudata*. These results suggest that shifts in flowering time and floral morphology have led to differentiation in pollinator assemblage between the two species.

Breeding systems

In the pollination experiments, the lack of fruit production in the bagging treatments indicated that the two species were dependent on insects for pollination, and spontaneous self-pollination did not occur in either species. Fruit set was significantly higher in the hand-pollinated treatments than in flowers visited by natural pollinators, suggesting that fruit production of both species in natural populations was pollinator-limited. Meanwhile, the seed set of all hand-pollinated and naturally pollinated individuals was relatively low in both species, suggesting that seed production in the two species was resource-limited.

In contrast to the high fruit set obtained from the crossing treatments (81.80% $\pm$ 4.97%), no fruit was found in the selfing treatments for *H. tenuiflorum*. These results suggest that the diploid species, *H. tenuiflorum*, was self-incompatible. However, in the pollen tube growth experiment, pollen grains started to germinate 4 h after hand-pollination and the pollen tube reached the ovary after 48 h (Fig. 3), indicating that there was no stigmatic or stylar self-incompatibility in *H. tenuiflorum*, and thus *H. tenuiflorum* exhibited post-zygotic self-incompatibility (Seavey & Bawa 1986; Sage & Sampson 2003). In comparison, the tetraploid species, *H. villosum*, was completely self-compatible; there was no significant difference in fruit set between selfing and crossing treatments for this species.

Polyploidy promotes wider spread

It has long been thought that polyploidisation might have allowed species to expand their range into novel environments as a result of derived ecophysiological differences relative to their diploid progenitors (Levin 2002; Ramsey & Schemske 2002; Soltis *et al.* 2009). *H. villosum* is widely distributed in Southeast Asia, including India, Myanmar, Thailand, Vietnam, Nepal and South China, at altitudes between 100 and 3400 m. However, *H. tenuiflorum* is only found in the Xishuangbanna area of South China and possibly in India at 800–900 m a.s.l. (Wu & Larsen 2000). The results of the investigation on distribution and habitat of these two species in China also confirmed that tetraploid *H. villosum* has a broader geographic distribution range and more diverse ecological habitats than diploid *H. tenuiflorum* (Yu *et al.* 2010).

The floral longevity of *H. villosum* is 5 days, which is longer than that *H. tenuiflorum* and much longer than other sympatric gingers (Gao *et al.* 2009). Floral longevity is assumed to reflect a balance between the benefit of increased pollination success and the cost of flower maintenance (Ashman & Schoen 1994, 1996). The results of manipulation experiments indicate that increased floral longevity is advantageous to both female and male fitness in *H. villosum* (Gao *et al.* 2009).

Polyploidisation results in a diversity of changes in floral traits, which enable *H. villosum* to exploit new habitats previously unsuitable for *H. tenuiflorum*. *Hedychium* is the only large Zingiberaceae that is widely distributed, from tropical Malaysia to the Himalayan highlands (Larsen *et al.* 1998). Polyploidy may play a very important role in the spread of *Hedychium* species from tropical areas to areas at high elevations.

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