The bracts of the alpine 'glasshouse' plant *Rheum alexandrae* (Polygonaceae) enhance reproductive fitness of its pollinating seed-consuming mutualist

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Environmental conditions are known to affect the reproductive fitness of insects, as do host plants. Although the highly specialized bracts of Himalayan 'glasshouse' plants are thought to be an adaptive morphology that allows the plant to reproduce in harsh alpine environments, little information appears to exist concerning the benefit of these bracts for the pollinating insects. In this study, we examine whether the semi-translucent bracts of *Rheum alexandrae*, a giant herb endemic to the alpine zone of the Hengduan Mountains in south-western China, enhance the reproductive fitness of its pollinator. *Rheum alexandrae* depends mainly on mutualistic seed-consuming *Bradysia* flies for pollination. Bracts increased interior temperature on sunny days, acted as a buffer against fluctuating air humidity, greatly decreased the intensities of ultraviolet B/C radiation and provided shelter from strong winds. Bract removal significantly decreased adult oviposition and offspring performance of pollinators during flowering and fruiting, respectively. Our results indicate that the bracts of *R. alexandrae* enhance the reproductive fitness of its pollinating seed-consuming mutualist in alpine environments, which may be attributed to the positive effects of the bracts on the interior microenvironment. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, **179**, 349–359.

ADDITIONAL KEYWORDS: insect activity – mutualism – offspring performance – oviposition – pollination.

INTRODUCTION

The alpine zone of the Hengduan Mountains region in south-western China is one of the most extreme terrestrial environments, characterized by low temperatures, high solar radiation, strong winds, frequent precipitation and a short growing season (Ohba, 1988; Yoshida, 2002). Plants in these areas frequently exhibit unusual specialized morphologies to allow them to reproduce in the hostile environmental conditions (Tsukaya & Tsuge, 2001). These conspicuous morphological adaptations are exemplified by Himalayan 'glasshouse' plants, the upper leaves of which have developed into large semi-translucent bracts that cover the inflorescences (Ohba, 1988). The glass-

house morphology has been recorded in more than ten plant families, including Ranunculaceae, Caryophyllaceae, Lamiaceae, Asteraceae and Polygonaceae (Yoshida, 2002) and has received substantial attention (Omori & Ohba, 1996; Omori, Takayama & Ohba, 2000; Yang & Sun, 2009; Zhang *et al.*, 2010; Liu *et al.*, 2013; Song *et al.*, 2013). The most prominent representative of the glasshouse plant species is *Rheum nobile* Hook.f. & Thomson (Polygonaceae). Previous studies suggested that the translucent bracts of *R. nobile* can warm interior reproductive organs of the plant and promote pollen germination and seed development (Omori & Ohba, 1999; Song *et al.*, 2013). In addition, the large bracts of *R. nobile* contain high levels of flavonol glycosides, which efficiently filter ultraviolet (UV) radiation and thus protect the reproductive organs from damage by the intense UV

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randomly assigned to the following two pollination treatments. (1) For autonomous self-pollination, four flower heads on each plant were marked randomly and these flower heads were covered by nylon mesh bags (mesh size = 10×10 threads cm^{-2}) throughout their flowering period to exclude pollinating insects. (2) For natural pollination, four flower heads on each plant were marked randomly and these flowers were not manipulated. When fruits were ripe, all marked flower heads were collected and taken to the laboratory to determine fruit set. Treatment 2 was also used to determine seed predation by counting the number of fruits with larvae or with hollowed-out fruit coats. These experiments were repeated in 2013.

EFFECTS OF BRACTS ON INTERIOR MICROENVIRONMENT

Temperature

Six plants were selected randomly during each of the flowering and fruiting stages to test the effects of bracts on interior temperature. The bracts in three of these plants were removed with scissors and the other three plants were left intact. Temperature of the flowers concealed by bracts and in the space between the bracts and flowers was measured using a four-channel thermocouple datalogger (Center 309) equipped with four alloy needle-type sensor probes (1–3 mm in diameter and with an active tip length of 5 mm); data were collected between 15 and 18 June 2012, and all days included sunny and rainy periods. Air temperature (*c*. 50 cm above the ground) was measured using an integrated thermistor (1400-104 air temperature sensors; LI-COR). Temperature was recorded at 5-min intervals. The measurements during fruiting were conducted between 13 and 16 August 2012, in the same way as during flowering.

Humidity

Five plants were selected randomly during each of the flowering and fruiting stages to test the effects of bracts on interior air humidity. The air humidity inside and outside (15 cm away from) the bracts was measured using an integrated thermistor (1400-104 air temperature sensors) under sunny and rainy conditions of the same day on 16 and 17 June, and on 15 and 16 August 2012 during flowering and fruiting, respectively. The measurements under rainy conditions started 20 min after rain beginning. In total, 80 measurements were conducted.

UV-B/C radiation

Five plants were selected randomly during each of the flowering and fruiting stages to test the effects of bracts on interior intensities of UV-B/C radiation. Intensities of UV-B/C radiation beneath bracts and in the open air were measured using UV-radiometers (Photoelectric Instrument Factory of Beijing Normal University) at 14:00 h on 16 and 17 June, and 15 and 16 August 2012 during flowering and fruiting, respectively. In total, 40 measurements for UV-B and 40 measurements for UV-C radiation were conducted.

Wind speed

Three plants were selected randomly to test the effects of bracts on interior wind speed during flowering. The wind speed inside and outside (1 m away from) the bracts was measured using an anemometer (Instrument Factory of Yunnan Normal University) from 08:00 to 18:00 h at 30-min intervals. Data were collected between 17 and 18 June 2012.

EFFECTS OF BRACTS ON ADULT ACTIVITY AND OVIPOSITION, AND OFFSPRING PERFORMANCE OF POLLINATING FLIES

To test the effects of bracts on pollinator activity, the number of flowers visited by individual female flies on plants with bracts or with bracts removed was recorded between 16 and 18 June 2012. The behaviour of individual flies was observed for 30 min and 20 flies each were recorded on plants with bracts and without bracts.

Twenty plants were selected randomly to test the effects of bracts on pollinator oviposition in 2012. Three flower heads on each plant were marked randomly, with the remaining flower heads carefully removed. We then randomly selected 20 flower buds on each flower head and the remaining flower buds were carefully removed. Nylon mesh bags (mesh size = 10×10 threads cm^{-2}) were used to exclude insects; hand-pollination was conducted once the stigma of selected buds became receptive. The next morning, these selected plants were divided into two groups. In the first group, the bracts were carefully folded back and held in place by thin wires, following the method of Song *et al*. (2013), thus preventing them being responsible for any effects during flowering; in the second group the bracts were left intact. Ten female flies collected from other flowering *R. alexandrae* were placed on each of the 20 selected plants and the plants were then bagged again. In the evening, the bracts in treatment 1 were unfolded carefully, and all flies in both treatments were removed. Four days later, all nylon mesh bags in both treatments were removed. When the fruits were ripe, all marked flower heads were collected and taken to the laboratory to count the number of fruits with fly larvae. We assume that the microenvironment experienced by the offspring developing during fruiting in the two treatments is identical. Therefore, we use the

proportion of fruits with fly larvae to estimate the effects of bracts on pollinator oviposition indirectly.

It is difficult to assess directly the effects of bracts on offspring performance of pollinating insects, so this was evaluated indirectly by recording the proportion of fruits with fly larvae, assuming that fly oviposition is randomly distributed among the plants. In 2012, 16 plants were selected randomly and three flower heads on each plant were marked randomly. After flowering, these selected plants were assigned to one of two groups. In the first group, the bracts were left intact; in the second group, the bracts were carefully folded back and held in place using thin wires, preventing them from being responsible for any effects during fruiting. When fruits were ripe, all marked flower heads were collected and we counted the number of fruits with fly larvae. These experiments were repeated in 2013.

DATA ANALYSIS

In this study, the experimental units were individual plants; accordingly, prior to statistical analysis we calculated means for plants from the measurements. Mixed model ANOVA was used to test the effects of measurement position, weather condition and developmental stage on air humidity and of measurement position and developmental stage on intensities of UV-B/C radiation. The data for different days were considered as repeated measures. Plants were a random factor nested within the developmental stage. The Kenward–Roger method was used to compute the denominator degrees of freedom. Independent-sample t tests were employed to test the effects of bracts on adult activity and oviposition of flies. To test the effects of pollination treatment and year on fruit set, and of bract treatment and year on offspring performance of flies, two-way ANOVA was employed. Data were $\log_{10}$-transformed if necessary. All analyses were performed with SAS statistical software (SAS Institute), with measured variables presented as means ± SE.

RESULTS

POLLINATOR OBSERVATIONS AND POLLINATION CONTRIBUTION

The fly fungus gnat *Bradysia* sp. was the only visitor to flowers of *R. alexandrae*, but the species is different from that pollinating *R. nobile* (Song *et al*., 2014). Female flies were observed foraging for pollen grains and laying eggs in the ovaries of parts of the flowers. When they crept on the flowers, pollen grains were attached to all parts of the body and could be transferred to the stigmas (Fig. S1D). At night, these female flies were observed to be inactive. No male flies were observed to visit the flowers.

Table 1. Two-way ANOVA on the effects of pollination treatment and year on fruit set of *Rheum alexandrae*

Effect	SS	d.f.	MS	F	P
Treatment	3.43	1	3.43	1596.77	<0.001
Year	0.008	1	0.008	3.69	0.16
Treatment × Year	0.001	1	0.001	0.59	0.45
Error	0.04	20	0.002		
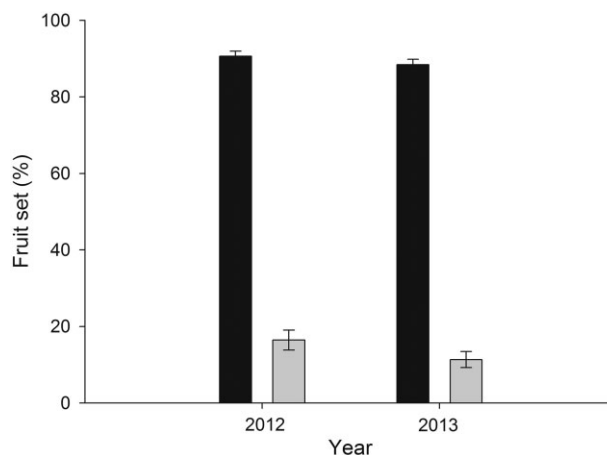
Figure 1. Fruit set (mean ± SE) of *Rheum alexandrae* flowers that were naturally pollinated (dark bars) and autonomously self-pollinated (bagged, grey bars) in 2012 and 2013.

Bagged flowers selfed autonomously, but fruit set was significantly reduced compared with that in open-pollinated flowers in both years (Table 1, Fig. 1). On average, fruit set resulting from natural pollination including pollinator visitation was 75.7% higher than for bagged flowers, suggesting that *R. alexandrae* depends mainly on *Bradysia* sp. for pollination. Fly larvae complete their development in the ripening seeds (Fig. S1E), resulting in 26.1 ± 0.6 and 30.7 ± 1.5% of the seeds consumed by the larvae in 2012 and 2013, respectively. The net pollination contribution of the fly to *R. alexandrae* was 47.3%, indicating that the interaction between *R. alexandrae* and *Bradysia* sp. is a pollinating seed-consuming mutualism.

EFFECTS OF BRACTS ON INTERIOR MICROENVIRONMENT

Temperature
During the flowering and fruiting stages, temperatures of flowers or ripening fruits concealed by bracts and in the spaces between bracts and flowers (or fruits) were up to 6 °C higher than conditions with
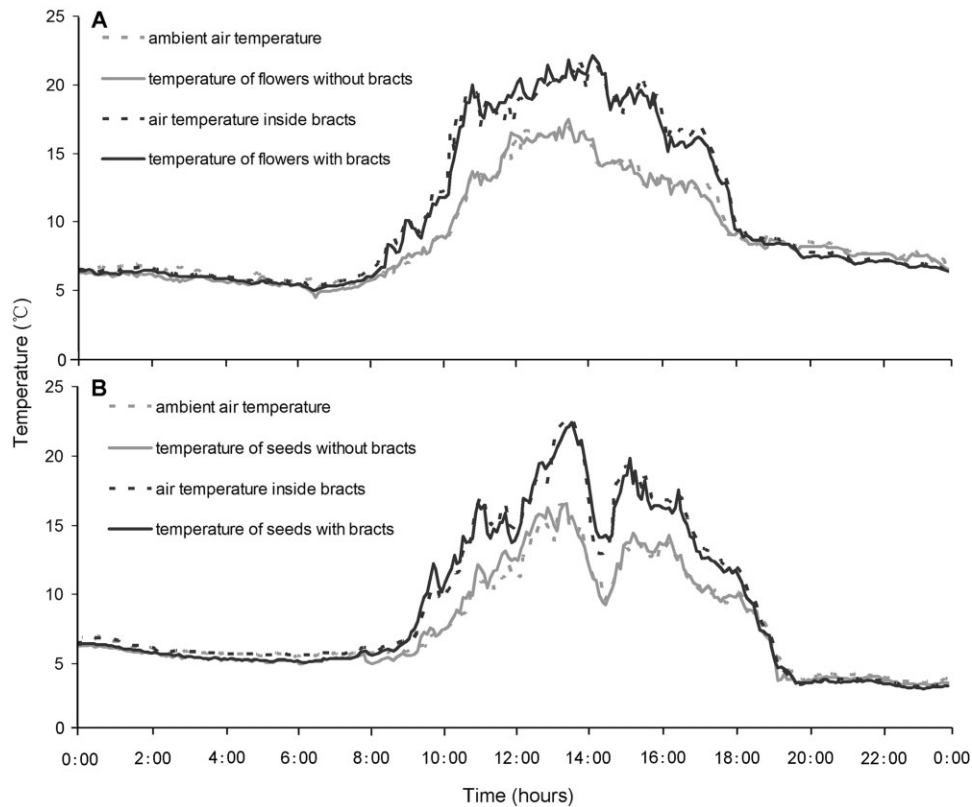
Figure 2. Variation in temperature in four different treatments during flowering (A, rain started at 14:10 h on 15 June 2012) and fruiting (B, rain started at 13:30 h on 14 August 2012).

Table 2. Mixed ANOVA on the effects of measurement position, weather condition and developmental stage on air humidity

Effect	SS	d.f.	MS	*F*	*P*
Stage	163.36	1	163.36	9.09	<0.01
Weather	21 733.82	1	21 733.82	1209.75	<0.001
Position	0.12	1	0.12	0.01	0.94
Stage × Weather	16.56	1	16.56	0.92	0.35
Stage × Position	47.37	1	47.37	2.64	0.11
Weather × Position	1956.24	1	1956.24	108.89	<0.001
Stage × Weather × Position	26.50	1	26.50	1.47	0.24
Plant (Stage)	182.11	8	22.76	1.27	0.31
Error	431.17	24	17.97		

bracts removed or than ambient conditions on sunny days (Fig. 2). However, rain and cloud obscured such temperature differences and night-time temperature differed little between treatments (Fig. 2).

Humidity
Air humidity was significantly higher during fruiting than during flowering (Table 2, Fig. 3). Weather condition strongly affected air humidity during flowering and fruiting, resulting in significant differences in air humidity between sunny days and rainy days, but the effect was disproportionately low inside the bracts (Table 2, Fig. 3). In fact, the differences in air humidity between sunny days and rainy days outside the bracts were 1.9 and 2.2 times higher than inside the bracts during flowering and fruiting, respectively.

UV-B/C radiation
There was no significant difference in intensities of both UV-B and UV-C radiation between stages
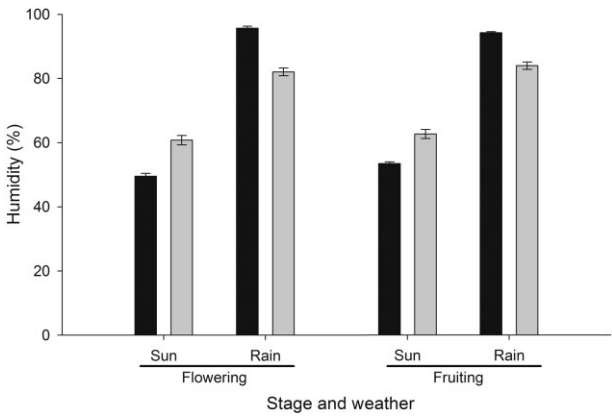
Figure 3. Relative humidity (mean ± SE) in open air (black bars) and inside bracts (grey bars) under sunny and rainy conditions during flowering and fruiting. Analysis of these data is presented in Table 1.

(Table 3, Fig. 4). Bracts significantly reduced the intensities of both UV-B and UV-C radiation reaching flowers and fruits (Fig. 4), and this effect was unaffected by developmental stage (Table 3). The intensities of UV-B and UV-C radiation for flowers (or fruits) concealed by bracts were only 1 and 20% of the ambient levels, respectively.

Wind speed
Wind speed was variable in the open air, ranging from 0 to 13.47 m s^{-1} and averaging 4.94 ± 0.18 m s^{-1}. However, there was no wind inside the bracts.

EFFECTS OF BRACTS ON ADULT ACTIVITY AND OVIPOSITION, AND OFFSPRING PERFORMANCE OF POLLINATING FLIES

The number of flowers visited by individual female flies within 30 min on plants with intact bracts was significantly higher than that on plants with their bracts removed (log$_{10}$-transformed data; $t = 9.16$, d.f. = 38, $P < 0.001$; Fig. 5), indicating that flies were more active in the presence of bracts.

The percentage of fruits with fly larvae on plants with bracts folded during flowering was significantly lower than that on the control plants (log$_{10}$-transformed data; $t = 4.60$, d.f. = 18, $P < 0.001$; Fig. 6), indicating that bracts had a positive effect on fly oviposition.

Bract removal by folding after flowering significantly reduced the percentage of fruits with fly larvae (Fig. 7), and this effect was consistent between years (Table 4). Compared with control plants, the percentage of fruits with fly larvae on plants with bracts removed after flowering decreased by 73.4% in 2012

Table 3. Mixed ANOVA on the effects of measurement position and developmental stage on intensities of UV-B/C radiation

Source	UV-B					UV-C				
	SS	d.f.	MS	F	P	SS	d.f.	MS	F	P
Stage	612.85	1	612.85	4.55	0.07	52 568.30	1	52 568.30	3.66	0.09
Position	137 080.78	1	137 080.78	1017.45	<0.001	10 918 891.54	1	10 918 891.54	760.98	<0.001
Stage × Position	584.54	1	584.54	4.34	0.07	24 564.91	1	24 564.91	1.71	0.23
Plant (Stage)	1130.20	8	141.28	1.05	0.47	238 806.35	8	29 850.79	2.08	0.16
Error	1077.84	8	134.73			114 787.40	8	14 348.42		
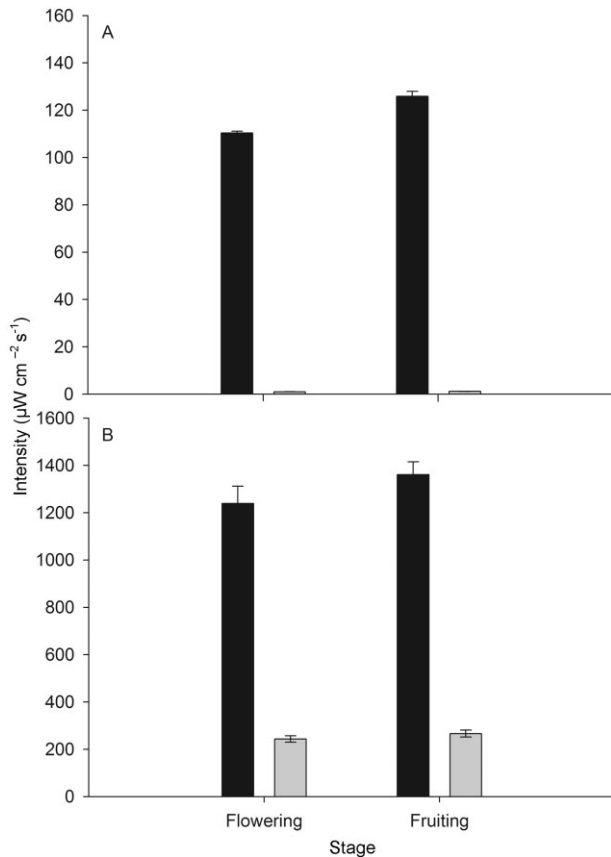
Figure 4. Intensity (mean ± SE) of UV-B radiation (A) and UV-C radiation (B) in open air (black bars) and beneath bracts (grey bars) during flowering and fruiting.
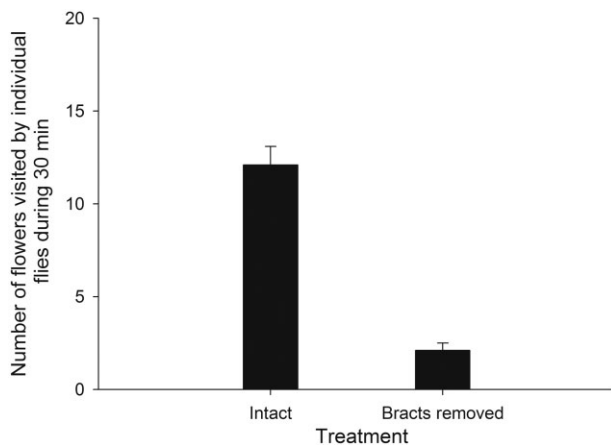
Figure 5. Number (mean ± SE) of *Rheum alexandrae* flowers visited by individual *Bradysia* sp. flies during 30 min under two bract treatments (intact vs. bracts removed).
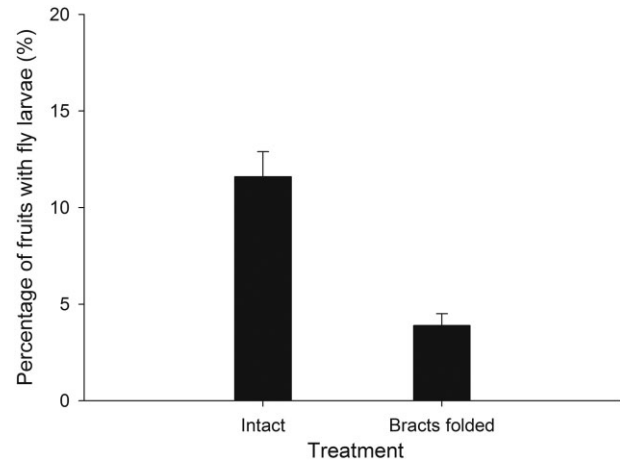
Figure 6. Percentage (mean ± SE) of *Rheum alexandrae* fruits with *Bradysia* sp. larvae under two bract treatments (intact vs. bracts folded back during flowering and unfolded during fruiting).
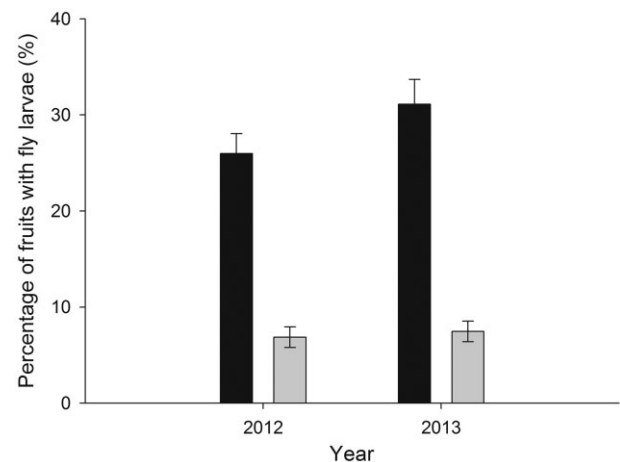
Figure 7. Percentage (mean ± SE) of *Rheum alexandrae* fruits with *Bradysia* sp. larvae under two bract treatments (intact: black bars; vs. bracts folded back after flowering: grey bars).

and by 75.9% in 2013, indicating that bracts enhanced fly offspring performance.

DISCUSSION

THE POSITIVE EFFECTS OF BRACTS ON POLLINATOR OVIPOSITION DURING FLOWERING

Our results showed that the percentage of fruits with fly larvae decreased by 66% for plants with bracts folded back during oviposition as compared with intact plants, suggesting that the bracts of *R. alexandrae* benefit the egg-laying activity of pollinating flies

Table 4. Two-way ANOVA on the effects of bract treatment and year on offspring performance of pollinating flies

Effect	SS	d.f.	MS	F	P
Treatment	0.37	1	0.37	137.58	<0.001
Year	0.007	1	0.007	2.49	1.26
Treatment × Year	0.004	1	0.004	1.55	0.22
Error	0.07	28			

during flowering. This interpretation of the effects of bracts on oviposition by flies could be questioned because, although folded bracts were unfolded after flowering, experimental folding of bracts may have had an impact on the interior microenvironment later during fruiting, which may have negatively influenced the offspring performance of flies. However, no significant differences in fruit set and seed weight were detected between the two treatments (B. Song, unpubl. data), suggesting the microenvironment during fruiting (i.e. during the offspring development of flies) for the two treatments is identical (Song *et al*., 2013) and thus the differences in the percentage of fruits with fly larvae can be attributed to the egg-laying stage. This was also validated by another of our experiments: bract removal resulted in an 88% reduction in the number of flowers visited by individual female flies within a 30-min period compared with intact plants. In fact, pollinating flies on plants with bracts removed were usually observed to be inactive and just rested on the flowers.

It has been demonstrated that poor weather conditions are disadvantageous to insect activities (Bergman *et al*., 1996; Vicens & Bosch, 2000). For insects, particularly ectothermic species, specific temperature thresholds are needed for activities (Morgan, 1987; Stone, 1993). For example, the oriental fruit fly, *Dacus dor salts*, is inactive at ambient temperatures below 16 °C (Roan, Flitters & Davis, 1954). Like the results reported for *R. nobile* and *Saussurea velutina* W.W.Smith (Asteraceae) (Yang & Sun, 2009; Song *et al*., 2013), the bracts of *R. alexandrae* can increase the interior temperature by as much as 6 °C compared with ambient air temperature on sunny days, which may provide a favourable thermal environment for egg-laying by pollinating flies. However, we found that the contribution of bracts to warming in *R. alexandrae* was less than by the bracts of *R. nobile*: the latter could increase the temperature of inflorescences by up to 10 °C over ambient levels on sunny days (Song *et al*., 2013). Meinzer & Goldstein (1985) suggested that larger plant organs may have a higher capacity to trap heat. Therefore, the difference in warming effects between the two species may result from the difference in size of the glasshouse structure

formed by the bracts: *R. nobile* is regarded as the tallest alpine herb in the Himalayan region and flowering individuals can reach a height of 1.5 m, whereas *R. alexandrae* commonly grows to a height of 0.8 m at most (Ohba, 1988; Li & Gao, 1998). For most insects, an appropriate relative humidity is important for activity (Bosch, 1994; Vicens & Bosch, 2000; Liu & Ye, 2006). Furthermore, high humidity may have significant negative effects on insect activity (Vicens & Bosch, 2000). For example, many insect species have been found to be active only at relative humidity < 90% (Vicens & Bosch, 2000). In particular on rainy days, insects are usually observed to stay in nests or shelters (Thatcher & Hertig, 1966). Some fly species have even been shown to be active only within a relatively narrow humidity range (Liu & Ye, 2006). *Rheum alexandrae* generally flowers from early June to early July, which coincides with the rainy season in the alpine zone of the Hengduan Mountains (Wang, 2006). In our study, the relative humidity inside the bracts of *R. alexandrae* fluctuated less than the ambient air humidity and, even on rainy days, the relative humidity inside the bracts was still < 90%. This can be explained by the fact that the tightly overlapping bracts are able to prevent convection between interior and ambient air. Accordingly, the presence of bracts in *R. alexandrae* may provide a favourable level of humidity for fly oviposition. Wind is also important for insect activity (Unwin & Corbet, 1991; Bergman *et al*., 1996). Vicens & Bosch (2000) found that even at a favourable temperature and relative humidity, moderate wind could cause the activity of muscoid flies to cease. Furthermore, high wind velocities can cause direct physical damage to insects. When a female fly is inserting her ovipositor into the style for oviposition on a plant without bracts, any strong wind may cause her lethal injury. The flowering season of *R. alexandrae* in the alpine zone of the Hengduan Mountains is characterized by frequent strong winds (Wang, 2006). Therefore, the presence of tightly overlapping bracts in *R. alexandrae* may be an important contribution to successful fly oviposition in alpine habitats. In addition to aforementioned direct effects, poor weather conditions are usually found to have synergistic effects on insect

activities (Liu & Ye, 2006). Consequently, our results indicate that the contribution of bracts to fly oviposition in *R. alexandrae* might be attributed to their positive effects on the interior microenvironment during flowering.

THE POSITIVE EFFECTS OF BRACTS ON POLLINATOR OFFSPRING PERFORMANCE DURING FRUITING

Our results showed that the percentage of fruits with fly larvae was significantly reduced on plants with bracts removed after flowering compared with intact plants, suggesting that bracts of *R. alexandrae* enhance the offspring performance of pollinating flies, similar to the results reported for *R. nobile* (Song *et al.*, 2013). It is well known that egg hatching and larval development for insect species need specific cumulative temperatures, to which physiological activities are sensitive (Gillespie, Sanchez & McGregor, 2004). Furthermore, temperature is also a crucial determinant of egg and larval development time (Williams, 1981; Grossmueller & Lederhouse, 1985). Tun-Lin, Burkot & Kay (2000) showed that the duration of development from egg deposition to adult for the mosquito *Aedes aegypti* (Diptera: Culicidae) was inversely related to temperature. Accordingly, heating associated with the presence of bracts in *R. alexandrae* during fruiting may help the egg and larva to develop faster in the ripening seeds. A shorter development time can enhance offspring survival in alpine environments with short growing seasons by allowing development to proceed to pupation before host plant senescence (Bonebrake *et al.*, 2010).

Egg-hatching and larval development of pollinating flies both occur between July and early September, when the UV radiation intensity tends to be highest in the alpine zone of the Hengduan Mountains (Wang, 2006). The embryonic stage of an insect is thought to be a period of UV radiation sensitivity (Tilton & Brower, 1983). Several studies have shown that intense UV (especially UV-B/C) radiation reduces egg hatching significantly, and the percentage of hatched eggs decreases with increasing duration of exposure (Guerra, Ouye & Bullock, 1968; Faruki *et al.*, 2007; Tuncbilek, Ercan & Canpolat, 2012). As Song *et al.* (2013) reported for the bracts of *R. nobile*, the bracts of *R. alexandrae* are able to screen UV radiation efficiently, and thus the intensities of UV-B and UV-C radiation reaching fruits concealed by bracts were found to be only 1 and 20% of the ambient levels, respectively. Thus, the bracts of *R. alexandrae* may protect fly offspring from damage by intense UV radiation. Our study showed that the bracts of *R. alexandrae* screen UV-B radiation more effectively than do bracts of *R. nobile*: the intensity of UV-B radiation reaching flowers concealed by bracts was

6.8% of the ambient level in *R. nobile*. This might result from the difference in their pigmentation: bracts of *R. nobile* are creamy white, whereas those of *R. alexandrae* are yellowish (Tsukaya, 2002). These results suggest that the bracts of *R. alexandrae* may be more specialized for protecting reproductive organs from UV radiation than the bracts of *R. nobile*. Further studies examining whether reproductive organs of *R. alexandrae* are more sensitive to UV radiation than those of *R. nobile* would be particularly useful for understanding the evolutionary significance of the specialized bracts.

Relative humidity can be one important determinant of egg hatching and larval development (Alyokhin *et al.*, 2001; Duyck, David & Quilici, 2006). For example, egg hatching rates of the fruit fly have been found to be reduced by 40 and 96% under slightly moist and drought conditions, respectively, compared with saturated conditions (Liu, 1981). Accordingly, the bracts of *R. alexandrae* may provide a favourable level of humidity for egg hatching of pollinating flies. It is also possible that the tightly overlapping bracts protect fly offspring from predation. However, we did not find any evidence of predation on the plants with bracts removed, indicating that bracts of *R. alexandrae* may not have an antipredator function. Therefore, the positive effects of bracts on the interior microenvironment during fruiting seems to be a plausible explanation for the enhanced offspring performance of pollinating flies compared with those on plants with bracts removed.

In conclusion, our results indicate that the bracts of *R. alexandrae* produce a 'greenhouse' effect similar to that reported earlier for *R. nobile* (Song *et al.*, 2013), positively affecting the interior microenvironment during flowering and fruiting: maintaining the interior warmth on sunny days, acting as a buffer against the ambient fluctuating relative humidity, screening UV radiation and providing shelter from strong winds. The positive effects of bracts on the interior microenvironment enhance adult oviposition during flowering and offspring performance during fruiting for the pollinating seed-consuming flies in the harsh alpine environmental conditions. Further study examining the responses of adult oviposition and offspring performance to these weather conditions individually or in combination using control experiments should be conducted to identify the key benefits of bracts associated with the reproductive fitness of the pollinators. Summarizing our present results in combination with the results reported for *R. nobile* (Song *et al.*, 2013, 2014), the nursery role of bracts for pollinating flies may play an important role in the evolution of the pollinating seed-consuming mutualism in this genus. Further studies on the pollination biology of other *Rheum* spp., combined with their

phylogenetic relationships, should be done to understand the evolution of plant–pollinator mutualism in this genus.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Rheum alexandrae and its pollinator. (A) A plant at the anthesis stage. (B) A plant in the middle stage of the seed development. (C) A plant in the late stage of seed development. (D) A female fly visiting a flower. (E) A fruit infested by a fly larva.