

Floral ecology of *Oreocharis pumila* (Gesneriaceae): a novel case of sigmoid corolla

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Attraction of pollinators and successful pollen transfer represent the primary targets of selection during flower evolution, leading to repeated evolutionary shifts between pollinators and consequently to the diversification of floral forms. However, most studies in floral evolution focus on the characteristics of flowers with straight corolla tube. Here, we report on an unusual form of sigmoid corolla combined with protandry and herkogamy in a Chinese species of Gesneriaceae, *Oreocharis pumila* (formerly *Opithandra pumila*). Contrary to species with sigmoid corollas studied previously, the base of the corolla tube of this species is inclined at an oblique angle downwards before the tube bends forward, and the stigma and anthers are included in the upper part of the corolla tube. The plants were found to be self-compatible but incapable of autonomous selfing. Successful pollination was found to depend fully on the presence of insect pollinators (*Nomia* sp.) and pollen grains are the greatest reward for the visitors. Different from the other sigmoid flowers, the sigmoid corolla of *O. pumila* was not found to favor insect pollinators with long flexible proboscises. A mechanical fit between floral morphology and pollinator was found, in which only small insect visitors with specialized visiting behavior are legitimate pollinators. The protandry combined with herkogamy in the sigmoid corolla tube strongly ensures pollination efficiencies. *Oreocharis pumila* is the only species with sigmoid corolla in the genus *Oreocharis*. We hypothesize that such a corolla has arisen through selection due to inadequate pollination in early spring in the mountainous habitat that *O. pumila* occupies.

Floral morphology is well known to affect the attractiveness of the plant to pollinators and the efficiency of pollen removal and deposition during pollinator visits (Stephenson 1979, Ehlers et al. 2002, Syafaruddin et al. 2006). In particular, changes in floral morphology that occur in primary sex organs (androecium and gynoecium) or in secondary sexual characters (e.g. inflorescence and corolla characters) can alter the plant–pollinator relationship and influence the dynamics of pollen removal and deposition (Quesada-Aguilar et al. 2008). Floral morphology that affects individual reproductive success may lead to evolutionary divergence and speciation (Herrera 1990). Therefore, attraction of pollinators and successful pollen transfer represent the primary targets of selection during flower evolution, leading to repeated evolutionary shifts between pollinators, and consequently to the diversification of floral forms (Harder and Barrett 2006, Alcantara and Lohmann 2010). However, most studies in floral evolution focused on the characteristics of flowers with straight corolla tube, such as floral color (Weiss 1995, 1997, Oberrath and Böhning-Gaese 1999, Tang and Huang 2010), corolla size and symmetry (Neal et al. 1998, Busch and Zachgo 2007, Gómez et al. 2008, Gómez and Perfectti 2010), and corolla tube length (Johnson and

Steiner 1997, Huang and Fenster 2007, Muchhala and Thomson 2009). In comparison, relatively little attention has so far been given to the function and evolution of sigmoid corollas (van der Pijl 1972, Stirton 1977, Potgieter et al. 1999, Potgieter and Edwards 2001, Potgieter et al. 2009).

Sigmoid corollas have been defined as corollas with varying degrees of geniculation of the tube (Codd 1985). They occur mainly among Lamiales, mostly in Lamiaceae and sporadically in Acanthaceae, Scrophulariaceae, Orobanchaceae, Lentibulariaceae, Gesneriaceae and Bignoniaceae. In general, the base of the corolla tube is held at a near vertical or oblique angle upwards before the tube bends downwards, and the anthers and stigma are situated at the mouth of corolla tube or protrude outside the corolla tube. Here, we report a previously unreported sigmoid corolla which was found in a species of Gesneriaceae, *Oreocharis pumila* (formerly *Opithandra pumila*, Möller et al. 2011), a small perennial herb endemic to Guangxi Province of south China (Wang et al. 1998, Li and Wang 2004). Contrary to the sigmoid corollae studied previously, the base of the corolla tube of *O. pumila* is inclined at an oblique angle downwards before the tube bends forwards, and both the anthers and stigma are included in the upper

part of the corolla tube. To our knowledge, *O. pumila* is the only species with sigmoid corolla tube in the genus *Oreocharis*. We think that our studies on floral biology of *O. pumila* will contribute to a better understanding of the biological function of sigmoid corollas, and flower specialization in *Oreocharis*. This study is aimed to address the floral ecology and breeding system of *O. pumila*, especially with the following questions: 1) What are the details of floral morphology and pollination ecology?, 2) Does a mechanical fit exist between floral morphology and pollinator?, 3) Does the sigmoid corolla of this species also favor landing insect pollinators with a flexible proboscis?

Material and methods

Species and study site

Oreocharis pumila (W. T. Wang) Mich. Möller & A. Weber was formerly known as *Opithandra pumila* (W. T. Wang) W. T. Wang, but recently included in *Oreocharis* (Möller et al. 2011). The plants are small, perennial acaulescent herbs growing on rocky outcrops in subtropical monsoon forests (Fig. 1A). The species is endemic to Guangxi Province of south China (Wang et al. 1990, 1998, Li and Wang 2004)

and flowers from February to April. The flowers are pink and broadly long tubular with a knee-like bend, and slight widening towards the zygomorphic limb (Fig. 1B). The two stamens are adnate to the adaxial side of the corolla tube, inserted in the base of the corolla tube (Fig. 1C), and the anthers cohere at the apex. The ovary is narrow cylindrical and glabrous, and the stigma is situated approximately at the middle of the corolla tube (i.e. the bend, Fig. 1C).

Our fieldwork was carried out during the flowering season in 2009 and 2010 on a population in Nalong in Xiaoming-Shan Mountain, Guangxi Province, south China (22°50.868'N, 107°40.542'E, 922 m a.s.l.).

Flowering phenology and floral morphology

In order to monitor the flowering phenology, 12–20 randomly selected flowering individuals were marked and observed from 8:00 to 17:00 h each day. For each flower, the length (position) of pistil (for stigma position) and stamens (for anther position), the time of anthesis, anther dehiscence and the flower wilting were observed and recorded at 8:00, 12:00 and 16:00 h every day. The number of inflorescences per individual, the number of flowers per inflorescence and newly opened flowers per individual were also recorded once a day. Additionally, the length and the

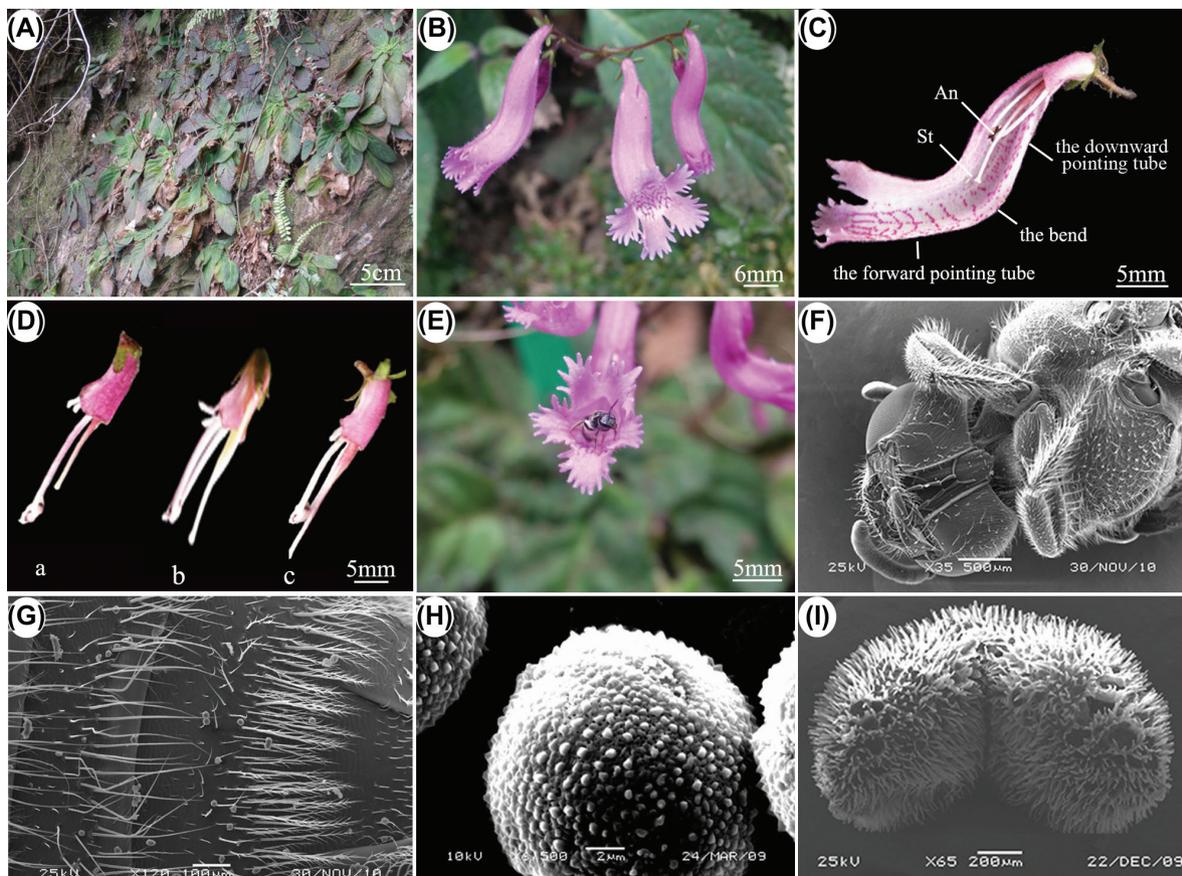


Figure 1. Habitat, flowers and their visitors of *Oreocharis pumila*. (A) habitat, (B) flowers and inflorescence, (C) day 3 flower with half the corolla tube cut off length wise, showing position relationship for stigma, anthers and sigmoid corolla tube; An = anthers, St = stigma, (D) flowers with corolla cut off in different development stages, showing position relationship between stigma and anthers; a = day 1 flower, b = day 2 flower, c = day 3 to day 6 flower, (E) *Nomia* sp. crawling out of the corolla tube, (F)–(G) ventral surface of *Nomia* sp., showing its head and abdomen–thorax carrying pollen grains (SEM), (H) pollen morphology (SEM), (I) stigma morphology (SEM).

diameter of the corolla tube were measured. Details of pollen and stigmas were later observed under a scanning electron microscope (samples were sputter-coated with gold-palladium). Pollen terminology follows Erdtman (1966).

Pollen viability, stigma receptivity, nectar production and pollen-ovule ratio

To test pollen viability, stigma receptivity, nectar volume and sugar concentration of this species, 10 fresh flowers bagged before anthesis from different plants were collected at 12:00 h every day during anthesis. Pollen viability was accessed by means of MTT (dimethylthiazol-diphenyl-tetrazolium bromide) (Dafni 1992). Pollen from 10 flowers in the same flowering period was mixed and five random samples of at least 500 pollen grains per sample were counted. Simultaneously, stigma receptivity was measured with a MTT test (Rodríguez-Riaño and Dafni 2000). Nectar was extracted with a 1 µl micro-capillary, and its sugar concentration (percentage sucrose, w/v) was tested with a handheld temperature-compensated refractometer (Brix 0–32%, ATAGO).

Pollen grains, ovules and pollen-ovule (P/O) ratios per flower were obtained by dissecting fresh flower buds prior to anthesis as described by Wang et al. (2004). Sixty fresh flower buds (1 or 2 d prior to anthesis) from different plants were collected and fixed in FAA (70% ethanol: formaldehyde solution: acetic acid = 90:5:5). Then, the anthers in each flower bud were soaked in 1M HCL for 3 h, squashed to release the pollen grains in a calibrated tube, and this was finally filled with 2 ml of staining solution. The suspension was vortexed for 60 s, and 10 separate 1 µl subsamples were transferred onto slides and the pollen grains were counted by using a haemocytometer with aid of a microscope. Simultaneously, ovules were counted by dissecting ovaries in each flower bud, and P/O ratios (number of pollen grains divided by number of ovules) was calculated for each flower.

Flower visitors and their behavior

We monitored flowers for insect visitors between 8:00–17:00 h on 27 Feb–1 Mar 2009 and 26–28 Feb 2010 for 1 h every 2 h per flower. Flower visitor behavior and their visiting frequencies were recorded. In addition to these observation periods, a lot of checks for flower visitors were also made during our studies. To assess whether a mechanical fit exists between floral morphology and the pollinator, we carefully cut a thin gap in the side of the corolla tube to observe visitor behavior. In order to determine whether the bend of the sigmoid corolla tube affects visiting behavior, we recorded and compared behavior and visiting frequency of all visitors between intact flowers and flowers with forward-pointing part of the tubes cut off. At least three individuals of each kind of visitor were captured for identification and observation under a scanning electron microscope.

Pollen removal and deposition

To test whether the sigmoid corolla tube influences pollen removal and pollen deposition, the pollen deposition on

stigmas and pollen removal from anthers were examined by collecting stigmas and anthers of intact flowers and flowers with forward-pointing part of the tubes cut off. These flowers were bagged before anthesis, and then exposed to open-pollination throughout the flowering period of the flower. Stigmas were embedded in a drop of aniline blue on microscope slides, and pollen grains were counted under a microscope at 20× magnification. Anthers were collected and the remaining pollen grains counted. The pollen-counting procedure was the same as used for counting pollen grains per flower. Pollen removal was determined by comparing remaining pollen with pollen produced in different flowers from the same plant individual (Dudash 1991). We also compared fruit set and seed set between intact flowers and flowers with a forward-pointing part of the tubes cut off.

Pollination experiments

To determine the breeding system, artificial pollination experiments were conducted and compared with open pollination in the population during 2009 and 2010. The following pollination experiments were performed and the fruit and seed set analyzed: 1) open pollination: flower buds were marked before anthesis and then left exposed; 2) autonomous autogamy: flower buds were bagged before anthesis until corolla withering to prevent cross pollination; 3) experimental autogamy: freshly opened flowers (day 2), which were bagged prior to anthesis, were self-pollinated by hand and then enclosed in bags until corolla withering to prevent cross pollination; 4) experimental xenogamy: pollen from plants at least 10 m away was placed directly onto the stigmas of freshly opened flowers (day 2), which were bagged before anthesis, and then enclosed in bags until corolla wilting to prevent geitonogamy.

Data analysis

Differences in visiting frequency, pollen deposition, pollen removal, fruit set and seed set (between intact flowers and flowers with forward-pointing tubes cut off) and the fruit and seed set (between out-crossed and selfed) were tested using independent-samples t-test. Differences in pollen viability among different development stages were compared with one-way ANOVA. All analyses of variation among treatments were performed in SPSS 11.5 and all means are presented with their standard error.

Results

Flowering phenology and floral morphology

The flowering period of *Oreocharis pumila* in the studied population was between mid-February to the end of April in both 2009 and 2010. Flowers of *O. pumila* usually open in the morning between 8:00–09:00 h and last 5–8 d (mean 5.83 ± 0.94 , $n = 12$). The plants produce 1–10 cymes per individual (mean 3.71 ± 1.97 , $n = 33$) with 1–5 flowers per inflorescence (mean 1.30 ± 2.68 , $n = 52$). There are

always 1–5 new open flowers per stem at one time (mean 1.84 ± 1.13 , $n = 33$).

Flowers of *O. pumila* are pink, with sigmoid shaped corolla tubes combined with a horizontal anterior corolla lobe (Fig. 1B), in which the base of the corolla tube is inclined at an oblique angle downwards and the front part of the tube is bent forward to present a horizontal opening. The length of forward-pointing tubes (from the corolla mouth to the bend of the tube) and downward-pointing tube (from the bend to the base of corolla tube) are 18.53 ± 2.50 mm ($n = 30$) and 17.75 ± 1.57 mm ($n = 30$) respectively. The width and height of both the forward-pointing and downward-pointing tubes are 6.19 ± 1.07 mm ($n = 30$) and 5.29 ± 0.88 mm ($n = 30$) respectively, but the width and height of the bend are 8.06 ± 0.88 mm ($n = 20$) and 4.87 ± 0.89 mm ($n = 20$) respectively. Both stigma and anthers are hidden in the corolla tube. The anthers are situated two thirds down the vertical tube during anthesis (Fig. 1C) and dehisce when the corolla opens. The pistil is shorter than the anthers at day 1 of flowering (Figs. 1D, 2), but elongates and protrudes beyond the anthers (herkogamy) from day 2 (Figs. 1D, 2). At day 3, the 2-lipped stigma with dense hairs (Fig. 1I) reaches the bend (Fig. 1C) and the pistil stops growing until flower withering (Fig. 2).

Pollen viability, stigma receptivity, nectar production and pollen-ovule ratio

Pollen grains of *O. pumila* are spheroidal with thorn eversion in their surfaces (Fig. 1H). The MTT test (Fig. 3) suggested that pollen viability was kept at a high level ($> 72.38\%$) before day 3 of anthesis, then significantly dropped to 53.51% at day 4 and only $21.16 \pm 5.67\%$ at the last day of anthesis ($F_{5,12} = 24.998$, $p = 0.0001$). There was non-receptivity of stigma in day 1 flowers (protandry), in which the pistil was below the stamens. From day 2, the stigma receptivity was 100% up to corolla withering.

Ovules and pollen production per flower were 679 ± 144 ($n = 60$) and $189\,657 \pm 50\,920$ ($n = 60$) respectively, and the resulting P/O ratio per flower was 295 ± 125 ($n = 60$). During anthesis, nectar volume produced in a flower was only 0.06 – 0.21 μl , with a sugar concentration of 5.12 – 6.45% .

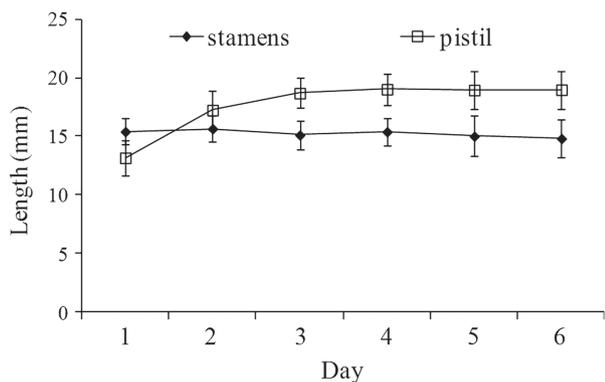


Figure 2. Length of stamens and pistil at flowers during anthesis (mean \pm SE, $n = 12$).

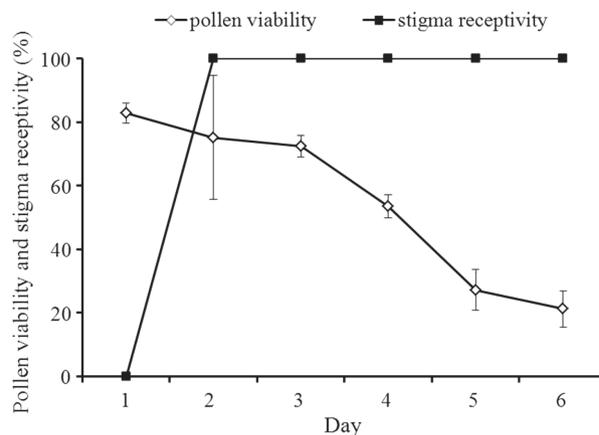


Figure 3. Pollen viability and stigma receptivity of flowers during anthesis (mean \pm SE, $n = 10$).

Flower visitors and their visiting behavior

According to our observations during the flowering seasons in 2009 and 2010, only two butterflies were recorded visiting flowers, which just occasionally landed on corollas without any contact with reproductive organs. A ground-nesting bee, *Nomia* sp. (Fig. 1E–G) was the most frequent visitor and legitimate pollinator. They usually visited flowers after 11:30 h during sunny days with a maximum visiting frequency of 0.22 times flower $^{-1} \cdot$ h $^{-1}$.

Nomia sp. landed on the lower corolla lip, and entered the flower on the dorsal side and crept along the corolla tube for foraging (Fig. 4A). At the bend of the sigmoid corolla tube, it turned its body upside-down and then continued to crawl along the downward pointing tube with their ventral side towards the anthers. In the day 1 flowers, the visitor got dusted with pollen from the anthers on their ventral surface while collecting pollen without touching the stigma, which is at this point unreceptive and positioned above the anthers. But from day 2 of flowering, the visitor would touch first the receptive stigma, which protrudes beyond the anthers, with its head and ventral surface depositing pollen (Figs. 1F–G, 4B–C) and then got to the anthers for foraging (Fig. 4D). At last, the small bee retreated with its ventral side upwards, and then turned around at the corolla bend (Fig. 4E) and crawled along and out of the corolla the same way it entered the corolla (Figs. 1E, 4F) that would result in self-pollination. The visitors were observed to bypass stigmas at the bend of the corolla tube for approaching anthers in the flowers with forward-pointing corolla tubes cut off. However, there was no significant difference in visiting frequency between intact flowers and flowers with forward-pointing corolla tubes cut off (Table 1).

The pollen removal and pollen deposition

Our result (Table 1) indicated that pollen deposition on stigmas of intact flowers was significantly higher than for flowers with forward-pointing tubes cut off ($t = 3.631$, $p = 0.001$). However, there was no difference in pollen removal between intact flowers and flowers with forward-pointing tubes cut

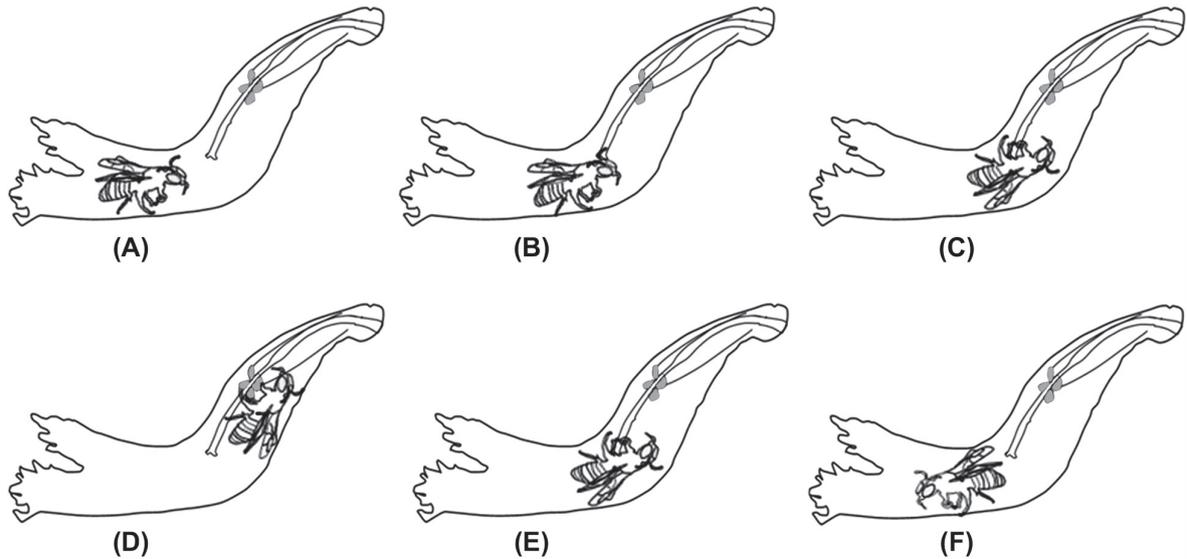


Figure 4. Mechanical fit between floral morphology of *Oreocharis pumila* and its pollinator (*Nomia* sp.). (A) visitor creeping into and along corolla tube, (B) visitor touching stigma at the bend with its head, (C) visitor turning its body upside-down at the bend and touching stigma with ventral surface (ventrally), (D) visitor getting dusted with pollen from anthers on their ventral surface and head by brushing movement, (E) visitor retreating with their ventral side upwards and overturning again at the bend, (F) visitor crawling along and out of corolla tube with belly downwards (dorsally).

off ($t = -1.586$, $p = 0.118$). Seed set of flowers with forward-pointing tubes cut off was significantly lower compared to intact flowers ($t = 8.833$, $p = 0.0001$), but there was no significant difference in fruit set between intact flowers and flowers with forward-pointing tubes cut off ($t = 2.757$, $p = 0.110$).

Breeding system

Our results (Table 2) showed that *O. pumila* is self-compatible and there was no significant difference in fruit set between self-pollination and outcross-pollination ($t = 0.376$, $p = 0.743$). The species is not apomictic or self-pollinating, as shown by the absence of seed set in flowers bagged before anthesis. Successful pollination clearly depends on the presence of pollinators.

Discussion

Angiosperm flowers vary in color, scent, size and shape, and these various floral characteristics are generally interpreted

as adaptations to attract one type of animal pollinator and to exclude other types (Crepet 1984, Suzuki et al. 2007). The optimal floral phenotype of animal pollinated plants (i.e. the floral phenotype with the highest reproductive success) should depend on the morphology and behavior of local pollinators (Ehlers et al. 2002), because successful transfer of pollen mostly rely on contact between the reproductive organs of a flower and its visitor in such a way that pollen is picked up on the visitor's body and deposited on the stigmatic surface of the host plant (Faegri and van der Pijl 1979). Species with extremely elongated corolla tubes might require specific pollinators of Lepidoptera or other insects with very long proboscides (Li 1951, Tang et al. 2007). The sigmoid tube of Lamiaceae (e.g. *Coleus* clade and *Plectranthus* clade) is always associated with a horizontal anterior corolla lobe, and this combination of characters is supposed to favor landing insects with flexible proboscis (Paton et al. 2004). The knee-like bend in the sigmoid corolla tubes (e.g. *Plectranthus laxiflorus*, *P. petiolaris* and *Pycnostachys urticifolia*) acts as a physical barrier that only allows insect groups with flexible proboscids of sufficient length to access nectar

Table 1. The visiting frequency of pollinator, removal and deposition of pollen, fruit- and seed set of *Oreocharis pumila* between the intact flowers and the flowers with upwards tubes cut off (mean \pm SE).

Treatments	Visiting frequency (times flower ⁻¹ h ⁻¹)	Pollen removal (pollen grain no. removed per flower)	Pollen deposition (pollen grain no. deposited per flower)	Fruit set (%)	Seed set (%)
Intact flowers	0.22 \pm 0.15 (n = 7)	173122.85 \pm 18063.85 (n = 36)	403.56 \pm 514.70 (n = 35)	21.08 \pm 5.97 (n = 172)	64.06 \pm 21.07 (n = 20)
Flowers with tubes cut off	0.15 \pm 0.11 (n = 7)	178436.00 \pm 3398.99 (n = 30)	53.13 \pm 126.96 (n = 30)	9.43 \pm 30.16 (n = 53)	4.26 \pm 3.64 (n = 10)
t	0.911	-1.586	3.631	2.757	8.833
p	0.366	0.118	0.001	0.110	0.0001

Table 2. Fruit set and seed set of *Oreocharis pumila* in different treatments (mean \pm SE).

Treatments	No. of flowers (plants)	Fruit set (%)	Seed set (%)
Open pollination	172 (20)	21.08 \pm 5.97	64.06 \pm 21.07
Autonomous autogamy	100 (17)	3.13 \pm 4.42	0
Experimental autogamy	79 (26)	16.60 \pm 16.41	25.32 \pm 31.79
Experimental xenogamy	104 (51)	25.81 \pm 30.49	35.74 \pm 22.27

(Potgieter et al. 2009). The sigmoid tube of *Oreocharis pumila* is also combined with a horizontal lower (anterior) corolla lobe favoring insects landing like on other sigmoid flowers. However, contrary to other sigmoid corolla tubes, the base of the *O. pumila* corolla tube is inclined at an oblique angle downwards and the front part of the tube is bent forward to present a horizontal opening. This means that it would not prevent the gravitational bleed of nectar which was thought as one evolutionary force responsible for the sigmoid corolla shape (Potgieter et al. 2009). Our result shows that *O. pumila* produces little nectar, and that pollen grains are the most important reward for visitors. The anthers and stigma of sigmoid corolla species are usually situated at the mouth of the corolla tube or protrude out of the corolla tube. After landing on the lower lip, the insect must angle its proboscis upwards into the corolla tube to access nectar at the base of the declined flower and is forced to contact the anthers and stigma which drop down into the boat-shaped lower lip (Potgieter et al. 2009). However, the anthers and stigma of *O. pumila* hide in the sigmoid corolla tube. In most animal-pollinated flowers, the organs, especially the corolla, are specialized with colors and scents to function as a pollinator attractant. Further specializations let the flower serve also as a pollinator manipulator, excluding organisms that would take nectar without effecting pollination and positioning a legitimate pollinator in a way to ensure pollen transfer (Kampny 1995). The sigmoid corolla shape limits the type and size of insects that can access nectar and act as pollinators (Meeuse 1992, Potgieter et al. 2009). Thus, large insects with long flexible proboscises cannot be effective pollinators of *O. pumila*, because the size of the narrow corolla tube excludes them from accessing pollen grains in the anthers at the base of corolla tube. Our study shows that the sigmoid corolla tube of *O. pumila* does not favor large insect pollinators with flexible proboscises. Only small insect visitors with specialized visiting behavior, which creep into the base of the narrow corolla tube for foraging, can be legitimate pollinators.

The sigmoid flower of *O. pumila* is an ingenious pollination device, in which the anthers are positioned at approximately two-thirds down the downward-pointing tube during anthesis, and the unreceptive stigma is below the anthers at day 1 of flowering (male phase). It makes the incoming small visitor to touch the anthers only, and not the stigma. From day 2 of flowering (hermaphroditic phase), the receptive stigma protrudes out of the anthers (approach herkogamy) and is situated at the bend of the sigmoid corolla tube at day 3 of flowering until corolla wilting. This

flower morphology induces an incoming visitor to touch the stigma first, hopefully depositing suitable pollen, and then get showered by the anthers (Kampny 1995). The protandry of *O. pumila* combined with the herkogamy approach in the sigmoid corolla tube strongly ensures pollination efficiencies, avoiding interference between male and female function. Our observation showed that visitors (*Nomia* sp.) would bypass the stigma at the bend and creep into the downward-pointing corolla tube to collect pollen grains in flowers with forward-pointing corolla tubes cut off. Our result also showed that pollen deposition and seed set in flowers with cut off corolla tubes were significantly less than for intact flowers. However, pollen removal did not differ between intact flowers and flowers with cut off corolla tubes. This indicates that the long horizontal part of the corolla tubes leads to increased protection of reward resources (nectar and pollen), which leads to increased pollinator fidelity (Potgieter and Edwards 2001). Often, the transition zone from the wide to the narrow part is of utmost importance for floral kinetics (Westerkamp and Claßen-Bockhoff 2007). It is here that the bend (8.06 \pm 0.88 mm) is the widest part of the sigmoid corolla tube of *O. pumila* and the width of the corolla tube is only 6.19 \pm 1.07 mm, but the size of the visitor *Nomia* sp. is 8.20 \pm 1.57 mm in length. Thus, this requires that the visitor turns its body upside-down only at the bend for foraging, leading to a fixed relationship between pollination surfaces of flower and visitor. Meeuse (1992) suggested that the size of specialized corollas and the resistance offered to insect visitors attempting to access nectar are important in corolla adaptations in Lamiaceae.

Oreocharis pumila is, as far as we know, the only sigmoid corolla species of the genus *Oreocharis*, which has experienced a rapid radiation early in its evolution and shows manifold convergences in floral characters (corolla form and coloration, fertility of stamens, anther shape and dehiscence mode) reflecting different pollination strategies (Möller et al. 2011). We hypothesize that the sigmoid corolla of *O. pumila* has probably arisen through selection due to inadequate pollination in its habitat. This species is always located at the peaks of mountains in south China and flowers in early spring. Inevitably, the plants suffer low temperatures, frequent strong winds and rainy weather during the flowering season, which constrains the abundance and activity of insect pollinators as is the case of alpine plants (Sieber et al. 2011). The sigmoid corolla of *O. pumila* with the stamens and pistil hidden in the corolla tube induces more specific behaviour of visitors to ensure that stigma and anthers function effectively. Previous studies have shown that species with very efficient pollination mechanisms have lower P/O ratios than species lacking these mechanisms (Cruden 1977, Wang et al. 2004). Our results also show that the P/O ratio of *O. pumila* is obviously lower in the facultative xenogamous flowers summarized by Cruden (1997).

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