

Flowers of *Cypripedium fargesii* (Orchidaceae) fool flat-footed flies (Platypezidae) by faking fungus-infected foliage

Zong-Xin Ren^{a,b}, De-Zhu Li^a, Peter Bernhardt^c, and Hong Wang^{a,1}

^aKey Laboratory of Biodiversity and Biogeography and Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China; ^bGraduate University of the Chinese Academy of Sciences, Beijing 100049, China; and ^cDepartment of Biology, Saint Louis University, St Louis, MO 63103

Edited by May R. Berenbaum, University of Illinois, Urbana, IL, and approved March 25, 2011 (received for review March 1, 2011)

Charles Darwin was fascinated by the orchid-pollinator interactions, but he did not realize that many orchid species are pollinated by deceit. *Cypripedium*, a model lineage of nonrewarding orchid flowers, is pollinated primarily by bees. Here we present both an example of floral mimicry of fungus-infected foliage in orchids and an example of flat-footed flies (*Agathomyia* sp.; Platypezidae) as pollen vectors for angiosperms. *Cypripedium fargesii* is a nectarless, terrestrial, endangered orchid from southwestern China that requires cross-pollination to produce the maximum number of viable embryos. All insects caught entering or leaving the labellum sac were *Agathomyia* sp. carrying conidia of *Cladosporium* sp. on their mouthparts and legs, suggesting mycophagy. Blackish hairy spots on the upper surface of foliage may imitate black mold spots, serving as short-term visual lures. Some odor molecules also associated with *Cladosporium* cultures were isolated in the floral scent. Mimicry of fungus-infected foliage probably represents an overlooked but important option in angiosperm diversification, because there are three to five more *Cypripedium* spp. in southwestern China with the same mode of floral presentation and black-spotted hairy leaves.

deceptive pollination | mimicry

Charles Darwin described and identified most of the functional floral morphology and biomechanics in orchid pollination without ever recognizing that many of the flowers that he examined lacked edible rewards (e.g., nectar, granular pollen) (1). In fact, floral evolution in the Orchidaceae appears to be dominated by modes of pollination by deceit (2), in which visual and/or olfactory cues mimic food sources, bodies of receptive females, and oviposition sites. Variation in floral mimicry appears to drive speciation in many lineages within the Orchidaceae (3–5).

The genus *Cypripedium* is regarded as a model lineage of food-deceptive orchids restricted to temperate regions of the Northern Hemisphere (6, 7). No *Cypripedium* flower studied to date has been found to secrete nectar or to offer accessible, granular pollen. However, observers continue to record insects entering their inflated labellum sacs (6), with the exception of three autonomously self-pollinating species (8, 9). The flower often produces a discernible and pleasant aroma, whereas the labellum is often a vivid and contrasting color from that of the broad, solitary staminode and the other five perianth segments. Insects that pollinate these flowers do not escape from the sac until they pass under the receptive stigma and then out through one of two rear apertures. This escape pathway forces the insect to contact one of two dehiscent anthers fixing either sticky, amorphous lumps of pollen or whole pollinia to the dorsum of the insect's thorax or head. With a few important exceptions (10, 11), bees of various sizes dominate the pollination of *Cypripedium* spp. (6, 7, 12, 13).

The biodiversity hotspot of the mountains of southwestern China is the center of diversity for this genus (8). It is here where

we find most of the small, single-flowered, geoflorous, dark dull-colored, unpleasantly scented species bearing labellum sculptures that resemble the surfaces of fungal sporocarps (14, 15). Vogel suggested that these species are pollinated by small flies, particularly fungus gnats (Sciaridae and Mycetophilidae) (13–16), but he was unable to prove it during his lifetime. Pollination by fungus gnats has been shown in some genera of monandrous orchids (4, 17), although some of these flowers mimic the bodies of female gnats, not mushrooms (2).

Here we focus on *Cypripedium fargesii*, a critically endangered species endemic to southwestern China (18), to elucidate its mechanism of floral mimicry and to interpret the function of black spots on its leaves. In this species, each short flowering stem bears two leaves with rows of black spots on the upper surfaces (Fig. 1A and B). The stem terminates in a small, solitary, dark-red to dull-yellow flower that produces a faint but unpleasant odor reminiscent of rotting leaves. We examined flower and leaf traits and conducted hand-pollination experiments to identify the breeding system. We tested embryo viability using a modified tetrazolium method (19). Pollinators were observed, captured by clogging floral apertures, and identified (20). Floral scent was collected outdoors by dynamic headspace adsorption methods and analyzed by GC-MS.

Results

The hand-pollination experiments showed that bagged controls never set fruit, but *C. fargesii* was self-compatible (Fig. 2), with fruit set rates for hand-mediated self- and cross-pollination of 80.0% and 77.3%, respectively, with no significant difference between treatments ($P > 0.05$, χ^2 test). The seed viability tests showed that cross-pollinated flowers produced significantly higher proportions of viable embryos compared with self-pollinated flowers ($t = 21.231$; $df = 6$; $P < 0.001$). Fruit set in flowers exposed to insects was low over four seasons (7.3%, 2.5%, 5.9%, and 3.3%, respectively; Fig. 2). This suggests that this population of *C. fargesii* is pollinator-limited, like most *Cypripedium* spp. (6).

As predicted by such low rates of insect-mediated fruit set, we saw few insects entering or escaping from these flowers despite 70 daytime and 15 nighttime h of observation at the field site. We collected and euthanized only three female and two male specimens in the orchids' labella or in the process of escaping from the interior of a labellum over a 4-y period. All specimens were flat-footed flies (*Agathomyia* sp., Platypezidae) (Fig. 1E). Specimen identification was based on morphology and confirmed by DNA barcoding. We observed that these flies entered and escaped from the labellum sac following the same

Author contributions: D.-Z.L., P.B., and H.W. designed research; Z.-X.R. and H.W. performed research; and Z.-X.R., D.-Z.L., P.B., and H.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: wanghong@mail.kib.ac.cn.