Plant Signaling & Behavior 9:11, e976484; December 1, 2014; © The Orchid Conservation and Research Center of Shenzhen

Orchid mating: the anther steps onto the stigma

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In a bisexual flower, the male and female organ of which have a space separation, individing into the pistil and stamen. Due to the spatial separation between male and female pollen grains from the anther of most flowering plants, including orchids, pollens are transported by wind or animals and deposited onto the receptive surface of the stigma of a different plant. Based on observations on floral morphology and flowering phenology, tests of the breeding system, and a comparison of pollination mechanisms, a new pollination process was discovered in the hermaphroditic (i.e., possessing spatially separated male and female organs) flower of a slipper orchid, *Paphiopedilum parishii*. The anther changes from a solid to a liquid state and directly steps onto the stigma surface without the aid of any pollinating agent or floral assembly. The mode of pollination in *Paphiopedilum parishii* is a new addition to the broad range of genetic and morphological mechanisms that have evolved in flowering plants to ensure their reproductive success. The present pollination mechanism is a possible adaptation to the insect-scarce habitat of the orchid.

Keywords: orchid mating, *Paphiopedilum parishii*, pollination mechanism, plant behavior, sexual organ liquefy

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Submitted: 08/18/2014

Revised: 08/25/2014

Accepted: 08/25/2014

http://dx.doi.org/10.4161/15592324.2014.976484

Addendum to: Chen LJ, Liu KW, Xiao XJ, Tsai WC, Hsiao YY, Huang J, Liu ZJ. The anther steps onto the stigma for self-fertilization in a slipper orchid. PLoS ONE 2012; 7:e37478

A self-mating story of a slipper orchid is emerging in southern China. Chinese scientists at the National Orchid Conservation Center in Shenzhen[1] displayed a rare orchid, *Paphiopedilum parishii* (**Fig. 1**) with its male organ releasing seminal fluid to female organ in a wild population.

In a bisexual flower, the male and female organs are separated into the pistil and stamen.[2,3] Due to the spatial separation between male and female organs, most pollination processes require the assistance of a pollinating agent, e.g., wind or gravity[4,5] and even animals,[6] to transport pollen grains from the anther to its own stigma.[4-6] Only a few species can overcome this obstacle in the floral structure. Such species move their floral components to achieve self-pollination in the late flowering phase. For example, stigma lobes bend toward a pollen-transferred surface, stamens bend toward a stigma,[7,8] or pollens slip toward the stigma with the aid of excreted oil from the anther and a specific groove on the stigma surface,[5] even selfing in the closed flower.[8,9]

Orchids usually separate the anther (stamen) and stigma (pistil) by a rostellum.[10] In some species such as *Paphiopedilum*, the anther even grows far from the stigma.[11,12] The labellum has also evolved to facilitate pollination by insects. Thus, orchids generally adopt a strategy of pollination by agent, or in the case of lacking pollinators, apply a mixed strategy of agent pollination[13] or autonomous self-pollination.[14-18] Some species develop a structure that interlinks pistils and stamens to assist the pollen in reaching the stigma and achieving self-pollination. For example, pollinia can enter the stigma cavity via the auto-spinning of the stipe. This type of autonomous self-pollination has been discovered in a study on the breeding system of *Holcoglossum amesianum.* In the wild, the pollinia of this species can rotate 360° via the auto-spinning of the stipe. As a result, the pollinia release pollen grains into the stigma cavity of the same flower.[4]

Slipper orchid *Paphiopedilum parishii* grows on tree trunks or rocks in broad-leaved forests. This species flowers from July to September during the rainy season. The flowers are 8–10 cm across with their

Figure 1. Flower of *Paphiopedilum parishii* orchid.

perianths dropping off, possessing the typical structure of the genus. When the flower of this species was about to open, the entire anther began to liquify and became pasty. The spreading-forward flower moved backward and upward. This movement adjusted the location of the anthers to the inclined top of the stigma when the flower fully opened from its original location. The liquified anther, which formed a liquid droplet, continuously liquified and amplified. It then gradually moved close to either lateral lobe of the stigma. As soon as the anther droplet made contact with the lobe edge, it stepped onto the papillate surface and rapidly spread onto the entire surface of stigma lobes.

The flowers of *P. parichii* were capable of self-fertilization by the automatic regulation of mating location as well as the automatic liquefaction and movement of the anther to span the space between the anther and stigma. No pollinating medium or agent is required, pollination occurs in open flowers, and the process is not a supplemental or back-up mode. The real novelty of this self-fertilization mechanism is that the anthers change to a liquid state. In *P. parishii*, there is nothing but a short distance exists between the anther and stigma. To achieve self-pollination, not only the pollen grains but also the anther itself liquefy and wholly step onto the stigma. The self-contained pollination mechanism is possibly an adaptation to unfavorable environments. Selfing by anther liquefaction and direct stepping onto the stigma from the anthers may have evolved as a strategy for coping with the scarcity of pollinators in the extremely shady and humid (insect-scarce[5]) habitats of *P. parishii*.[5,19,20] Given that crossing can not occur without pollinators, *P. parishii* evolved a self-breeding strategy for species survival.

Paphiopedilum parishii has evolved its "anther steps onto the stigma" feature, a peculiar self-pollination mechanism that is a successful evolution indicating that the reproductive assurance of selfing has greater function than inbreeding depression during the evolution of facilitating mating system in plants. We should not underestimate the ability of plants to cope with unfavorable habitats. We should give importance to research on the effects of selfing mechanisms on plant survival.

Slipper orchids are generally cross-pollinated, a strategy of needing the aid of insect pollinators. Based on the flower structure, this slipper orchid is cross-pollinated, as found in most species of the genus, but in its natural environment in southern Yunnan, the insects are very rare. Even though the plants are "willing" to carry out self-pollination, it is difficult for them to obtain a touch or a shake caused by either insect or wind. What is more, the male and female organs of this species are almost at the same horizontal level. Probably, as a result, the orchid adopted a new pollination strategy to deal with such a situation.

The solid-state anther can automatically liquefy, and the whole liquefied anther can step from the apex of the filament onto the stigma to achieve self-fertilization. To the best of our knowledge, this process is a newly discovered mode of pollination in plant reported for the first time. The present self-contained pollination is a new self-pollination mechanism in orchids and even in angiosperms, and is like to be an adaptation to the insect-scarce habitat. This maybe is a continuation of the Darwin's orchid story.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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