

# REPRODUCTIVE BIOLOGY OF A MEDICINAL ORCHID *SATYRIUM NEPALENSE* (ORCHIDACEAE) IN THE NEPALESE HIMALAYAS

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## ABSTRACT

*Satyrium nepalense* is a terrestrial medicinal orchid of the Himalayas. Although, the ecology and medicinal uses of this orchid is well known, little is known about its reproductive mechanism. In this study, we explored the natural history and reproductive biology of *S. nepalense* and tested their potential spatial variation. We observed the floral visitors and natural fruit set of *S. nepalense* at its two populations. We found that, despite the presence of outcrossing features, this orchid did not receive any diurnal floral visitors. The natural fruit set was very high (>90.0%) at both the sites, suggesting the possibility of autonomous selfing. This finding may have important implication for the further study to explore how autonomous selfing evolves in a member of the predominately outcrossing genus. Moreover, the finding of this study provides important insight to develop efficient protocol for its conservation.

**Key words:** Autonomous selfing, conservation, floral traits, orchids, pollination

## INTRODUCTION

Orchids, the members of the family Orchidaceae, are well known for possessing exquisite flowers exhibiting great diversity in shape, size, color, fragrance, and texture (Dressler, 1981). This diversity in floral architecture is often considered the result of adaptation towards different pollination mechanisms (Darwin, 1862; Van der Cingel, 2001). Accordingly, they exhibit complex pollination strategies including various curious mechanisms of autonomous selfing, specialized biotic pollination assisted either by reward or mimicry leading to deceptive pollination, and/or various forms of abiotic pollination (Johnson, 1996; Johnson *et al.*, 2011). However, most orchids evolve mechanism to prevent self-pollination and thus reproduce through a highly specialized pollinator mediated outcrossing. Thus, the natural reproduction

in orchids often relies upon the availability of pollinators (Catling 1990; Peter 2009). This high level of pollinator specialization may often lead to reproductive failure if the phenology/ distribution/ habitat of pollinators are disturbed by multifarious factors including anthropogenic disturbances. In alpine habitat, the availability of pollinator is limited by several factors and thus the alpine orchids may suffer from high level of pollen limitation (Catling, 1990, Johnson and Steiner, 2003; Tremblay *et al.*, 2005). As a result, like other alpine plants, orchids in the high Himalayas may evolve either abiotic pollination or autonomous selfing to assure their reproductive success. Studies have shown that *ca* 400 orchid (comprising *ca* 5% orchid) species are known to reproduce via autonomous selfing while the cases of abiotic pollination are rare (Catling, 1990; Peter, 2009). Due to these

specific pollination system of orchids, exploring the reproductive mechanism is an essential step in developing efficient protocol for their conservation.

The genus *Satyrium*, a twin-spurred orchid, is well known for possessing unsurpassed floral diversity particularly associated with pollinator mediated pollination systems (Van der Niet *et al.*, 2005; Liltved and Johnson, 2012). Consistent with the floral traits, most members of the genus exhibit specialized biotic pollination system including bee, beetle, bird, butterfly, fly, and moth as the specialized pollinators (Johnson, 1996, 1997; Johnson *et al.*, 2011). *Satyrium nepalense* D. Don like other members of the genus possesses the floral traits that are consistent with pollinator mediated pollination system. Thus, we anticipate pollinator mediated breeding system in this orchid. Although, the ecology and medicinal uses of this orchid have been studied by several authors, its reproductive biology is yet unexplored (Mishra and Saklani, 2012; Paudel and Poudel, 2019). In this study, we explored the natural history and reproductive biology of *S. nepalense* and tested their potential spatial variation.

## MATERIALS AND METHODS

### Study species and study sites

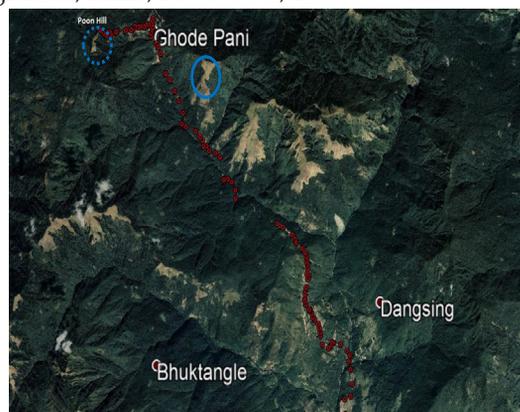
*Satyrium nepalense* is a terrestrial orchid distributed from the Indian subcontinent to South central China. In Nepal, it is distributed across the central, western and eastern parts of Nepal between the elevation of 1000-3200 m (Raskoti, 2009). It preferably grows in south/east facing open meadows (Paudel and Poudel, 2019). It is a medium sized herb with 14-32 cm height, cylindrical stem and possesses 2-3 sheathing with sheath leaves. Flowering occurs from August to October with the peak blooming in early September. An individual plant produces a single erect spike (inflorescence) that

usually bears 10-50 compactly arranged rose pink flowers (Fig.1) (Raskoti, 2009).



**Figure 1: A flowering individual of *S. nepalense* at its natural habitat (Deorali Pass)**

The study was conducted at two sites within the Annapurna conservation area. The first site (Deorali Pass) is a south facing meadow located just above Nangethati, Kaski while the second site is an east facing meadow close to the famous tourist destination Poonhill, Myagdi (Fig. 1). At both the sites, the plant grows mixed with another terrestrial orchid *Herminium* sp., several grasses, ferns, *Pedicularis*, *Potentilla* etc.



**Figure 2: Study sites. Site included in solid circle indicates Deorali Pass while the site included within dotted circle indicates Poonhill area. Dotted red line indicates the trekking route to the study sites.**

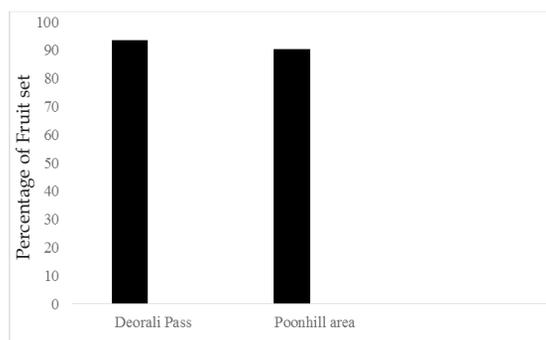
### Breeding system experiments

At each sites, we observed the potential visitors to *S. nepalense* flowers for three consecutive days

from 7:00 to 17:00 hr. As this time interval is the peak foraging time of diurnal visitors, this observation includes all the potential diurnal floral visitors. Due to logistic constrain, however, we were unable to observe the potential nocturnal visitors. To determine the potential mechanism of natural breeding system, at each site we randomly selected 25 flowering individuals. All the flowers of the experimental individuals were then counted, tagged, and left intact to allow fruit set. After about a month, fruits formed in each experimental individual were separately counted, and the percentage of fruit formed in each individual plant was assessed. We then tested the differences in fruit set percentage among the different plants at a site. Finally, the differences in fruit set percentage between the two sites were assessed. We used generalized linear models with binary distribution of errors to differentiate the fruit set percentage among the individual plants/between the sites. All the analyses were performed in R package (version 3.3.1) (R Core Team, 2015).

## RESULTS

Our result revealed the absence of diurnal visitors to the flowers of *S. nepalense*, i.e., during our three days' survey at each site, we did not observe any visitors to the flowers of *S. nepalense*. We found that at both the sites more than 90% flowers at each inflorescence (individual plant) formed fruits. Our analysis revealed that the fruit set percentage among the different plants (inflorescences) within a site did not differ significantly ( $P > 0.05$ ). Similarly, the fruit set percentage did not differ significantly between the two sites ( $P > 0.05$ ). The average fruit set percentages at Deorali pass and Poonhill area were 93.45% and 90.43% respectively (Fig. 3).



**Figure 3: Natural fruit set percentage of *S. nepalense* at the two experimental sites.**

## DISCUSSION

Our result indicates that despite the presence of several outcrossing floral features, *S. nepalense* flowers lack diurnal floral visitors, yet the natural fruit set is very high. The high proportion of fruit set data suggest the possibility of autonomous selfing in this orchid. Although the fruit set success in this orchid could have been either via the contribution of nocturnal pollinator or via abiotic pollination mediated by air and water. However, our data does not support for either of the possibility. Under the condition of nocturnal pollination, usually the fruit set proportion could not be as high as the current data. Similarly, abiotic pollination assisted by wind or water also seems unlikely to occur because of the aggregation of pollen grains into pollinia that are not easily dislodged by rain and/or water (Suetsugu, 2019). Therefore, the reproduction in *S. nepalense* seems to occur through autonomous selfing. Our finding is consistent with the finding of Niet (2018) in *S. rupestre*. Given the absence of autonomous selfing in most members of the genus, and the presence of outcrossing floral feature, why and how *S. nepalense* evolve autonomous selfing is an intriguing question for further research. Niet (2018) suggests that the presence of unusual column in the flowers facilitates autonomous selfing in *S. rupestre*. So,

it suggests that *S. nepalense* may possess such a unique floral feature that facilitates autonomous selfing. However, our current limited data cannot provide strong evidence in favour of autonomous selfing in *S. nepalense*. Therefore, further study including the close observation of floral traits and manipulated pollination experiment would be required to shed light in the actual pollination mechanism of this highly valued medicinal orchid of the Himalayas.

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