Original Article

Current Botany 2017, 8: 149-154
http://scienceflora.org/journals/index.php/jp/
doi: 10.19071/cb.2017.v8.3256

Impact of floral density on pollination success rate and fruit set of *Calceolaria gracilis* Kunth in Darjeeling Himalaya

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Received: 21.06.2017
Accepted: 05.09.2017
Published: 26.09.2017

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ABSTRACT

The present investigation deals with the impact of floral density on pollen biology with reference to pollen production, pollen-ovule ratio, pollen viability, and pollen fertility in the context of pollination success rate (PSR) and fruit set of *Calceolaria gracilis* Kunth of the family *Calceolariaceae* in Darjeeling Himalaya. It has been observed that there are positive correlations between the density of the flowers and pollen biological features such as the productivity of pollens, pollen-ovules ratios, and viability and fertility of pollen grains. Fruit set (%) becomes higher having highly dense floral site than low ones due to higher pollen fertility rate, low sterility, and higher number of pollen load upon stigma. It is concluded that floral density exerts noticeable effects on the PSR and fruit set in terms of reproductive fitness of this species although it is suggested that at least four different tests for pollen viability including *in vitro* germination test of pollen grains are yet to be studied.

KEY WORDS: Angiosperms, pollen fertility, pollen viability, pollination success

INTRODUCTION

Pollination and pollen biology is normally considered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac in a compatible manner. Assessment of pollen biology with regard to its viability on the basis of its function is cumbersome, time-consuming, and not always feasible. Pollen viability has been evaluated by various staining techniques such as tetrazolium salts (triphenyl tetrazolium chloride) to detect dehydrogenase activity, fluorescein diacetate to determine esterase activity, by *in vitro* and *in vivo* pollen germination tests. The choice of method may depend on the type of species. There has been very little information about the utility of most of these methods for *Calceolaria gracilis* of the family *Calceolariaceae* growing in the vicinity of Darjeeling Himalaya. Sexual reproduction and the associated exchange of genes seem to be essential for population survival of most flowering plants. If gene flow between plant populations is low it will cause inbreeding and poor adaptation to a changing environment eventually leading to extinction. Studying the role relative floral density in mediating pollination services can serve as a model for understanding ecological processes at different stages of reproduction. Pollinator foraging behavior depends on processes operating at the level of sites of floral resources within a broader floral landscape and processes within the site. If attraction to larger sites of floral resources is an accelerating function of floral density, facilitation of pollinator visits to plant species within the site is possible (Feldman *et al.,* 2004). Total floral density and floral diversity affect pollinator choice among sites, whereas relative floral density should affect pollinator foraging decisions among floral resources within a site (Ghazoul, 2006). Greenleaf *et al*. (2007) suggested that flower density shows a positive correlation between pollination success rate (PSR) and seed set in flowering plants whereas density and abundance of flowers as well as plants might have an impact upon seed set and pollination success of many taxa (Dietzsch *et al*., 2011; Yang *et al*., 2011). Hanoteaux *et al*. (2013) proved the effects of spatial patterns on the pollination success of a less attractive species upon its fruit set and reproductive success rate. The present work has been carried out to know the role of plant and floral density upon the pollen biological features such as pollen production and ovule ratio, pollen viability, sterility, fertility, and PSR in terms of fruit-set of *C. gracilis* belonging to the family *Calceolariaceae* growing in Darjeeling Himalaya as there are very little or scarce documentation on such type of investigation based on this species.

MATERIALS AND METHODS

Darjeeling (27°13″ N to 26°27″ N latitude; 88°53″ E to 87°59″ E longitude; 3149 km - area; 6710 feet altitude; annual mean max. temperature 14.9°C; annual mean minimum temperature 8.9°C and average annual rainfall 3092 mm) hill areas is unique from environmental eco-perception. The relief varies from 100 m above sea level to the mighty Kanchenjunga. There are different climatic zones with distinctive attributes and there are many plants and pollinators in this hilly region. The Hill areas of Darjeeling District are located within the lesser and Sub-Himalayan belts of the Eastern Himalayas. The area is bounded by the Sikkim Himalaya in the north, the Bhutan Himalaya in the east, and Nepal Himalaya in the west. The southern foothill belt is demarcated by a highly dissipated platform of terrace deposits extending along the east-west axis. The inner belt is defined by a ridgeline stretching from the Darjeeling Hill to the west and Kalimpong Hill to the east, overlooking the southerly flowing Tista Valley in between. Prominent rivulets contributing to the Rammam-Rangit basin, dissipate the northern slope of Darjeeling Hills. The forests in and around Darjeeling have delightful flora and fauna.

C. gracilis is a terrestrial, erect, shrub with terete, densely puberulous, glabrous, greenish purple in color stem. The leaves are stipulate 5 mm × 3mm; opposite superposed in phyllotaxy; compound, lamina ovate to ovate lanceolate 19 mm × 8 mm × 44 mm × 20 mm; crenate lobulated with 5-7 pairs of denticulate lobules at margin (each lobule again having serrulate margin), blunt acuminate to acute apex, sparsely puberescent abaxially but densely pubescent adaxially; conspicuously unicostrate or brachidodromous type venation with 4-6 pairs of lateral veins; venation- cercinate, lobed at base. Petiole-8-16 mm. long, soft beset, with dense pubescent hairs, and flat. Inflorescence: Axillary as well as bracteate cymes, covered with two bracteoles. Flower: Zygomorphic, pentamerous Ca. 19 mm long bright yellow with bracts and bracteoles. Bracts - two leaf such as green, ovate Ca. 6 mm acute at apex, serrulate ciliate at margin, cuneate at base and hairs on both surfaces, Bracteoles - two, at basal green membranous, elliptic, lanceolate, and minutely puberulous. Calyx: Cup-shaped Ca. 8 mm long; 4 lobed, oblong lanceolate, 6 mm long and 4 mm wide, light green, shortly acuminate, ciliolate at margin, and puberulous. Corolla: Bilabiate, bright yellow Ca. 18 mmlong, upper part 3 lobed, flat, larger, oblong, connate forming bag such as structure, pilose, each lobe rounded at apex with dense cillia, inside and outside glabrous; lower corolla 2 lobed Ca. 3 mm both outside and inside glabrous. Androecium: Epipetalous, stamens 2, Ca. 5 mm long, yellowish white; filaments yellow, Ca. 4 mm long, glabrous; anther lobes 2 celled, smooth, Ca. 1 mm long flate - obovate, longitudinal dehiscence, below female part. Gynoecium: Carpels 2, Ca. 4 mm, ovary oblong obovate, Ca. 2 mm long, glabrous, biloculer, syncarpous, each locules with numerous ovules on axile placentation; style filiform Ca. 1 mm long, glabrous; stigma capitate with numerous unicellular papillae cells. Fruit: 11 mm long, sicula type. Floral biology, i.e., flower anthesis, pollen anthesis, pollen production, and pollen-ovule ratio estimation was studied at two sites (Darjeeling Town and Jorebunglow) following the procedure suggested by different scientists in the concerned field of research (Mathur and Ram, 1986; Bhattacharya and Mandal, 2004). Insect behavior was studied according to Faegri and Pijl (1981). Pollen sterility, pollen viability, and fruit set were investigated following the method suggested by Shivanna and Rangaswamy (1993). The statistical analysis was performed using the software SPSS version 10.1.

RESULTS

A considerable effect of floral density and exposure were observed on pollen biology in terms of pollen production, log pollen estimation, pollen-ovule ratio estimation, pollen viability, fertility, sterility, and fruit set of selected species (Table 1). Field experiments through netting and bagging of flowers were carried out on this small plant (Figures 1-3). Pollen production and fruit set were found to be higher in open/control flowers compared to netted and bagged flowers (Figures 4 and 5). No good impact of netting and bagging was found on pollen viability although fertility and sterility of pollens seem to be dependent greatly on flower exposure (Figures 6-8). A great variation of pollen-ovule ratio was noticed in unexposed through netting and bagging flowers versus exposed or open flowers (Figure 9). Pollen load on stigma and number of papillae cells on stigmatic surface are also variable with flower exposure to environment (Figures 10 and 11). Higher the density of flowers higher the productivity and viability of pollens and fruit set percentage, whereas, lowers the pollen-ovule ratio (Figures 12-15) although sterility and fertility of pollens are not greatly dependent upon flower density (Figures 16 and 17).

DISCUSSION

The pollination mechanisms and pollen biological features might be influenced by different factors such as floral density,

Table 1: Floral biological features of *C. gracilis*

Observations	Darjeeling town	Jorebunglow
Density of plant/m^2	12.2±0.69	9.2±0.75
Density of flowers	4.6±0.29	3.8±0.54
Pollinators profile	*Vespa* sp.	*Vespa* sp.
Flower anthesis (h)	6:00-6.30 a.m.	6.00-6.30 a.m.
Pollen production/anther	3176	2995
Pollen production/flower	6332	6180
Number of ovules/flower	188	145
Pollen ovule ratio/lower	38:1	45:1
Pollen viability (%)	74.94±1.92	58.10±3.44
Pollen fertility (%)	75.76±1.12	69.55±2.55
Pollen sterility (%)	27.45±0.45	33%±1.17
Pollen load on stigma	138±11.32	25.5±10.16
Number of papillae cells on stigma	47±2.92	29±2.28
Fruit set (%)	7.94±1.18	4.83±0.27

C. gracilis: Calceolaria gracilis

Figure 1: Habit of plant

Figure 2: Bagging

time of flower and pollen anthesis, temperature, humidity, seasonal effect, flower position, anther protection, age of pollen, pollinator type, pollen packaging, number of nucleus, carbohydrate content, and desiccation risk with reference to pollen metabolism and genetic variability. After release

Figure 3: Netting

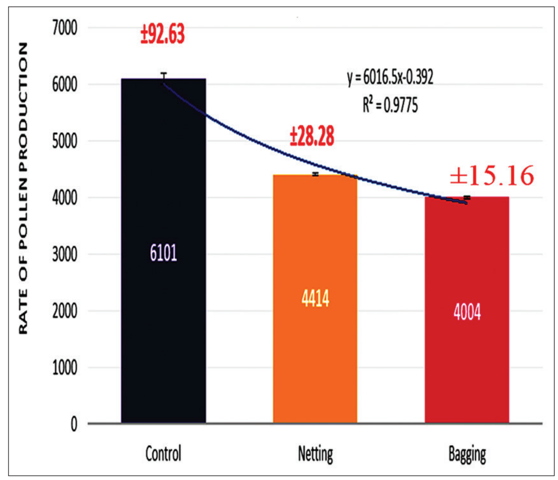

Figure 4: Effect of netting and bagging on pollen production/flower

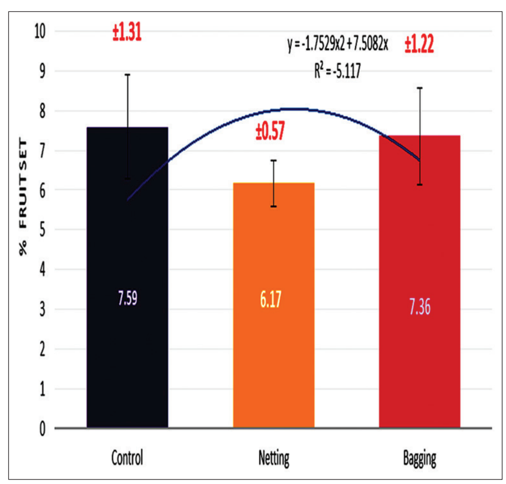

Figure 5: Effect of netting and bagging on fruit set

from the anthers, the pollens are exposed to potentially hostile environments, such as dry or humid conditions, canopy gap, pollinators, and other biotic factors, and yet they perform well and germinate to pollinate the stigma and

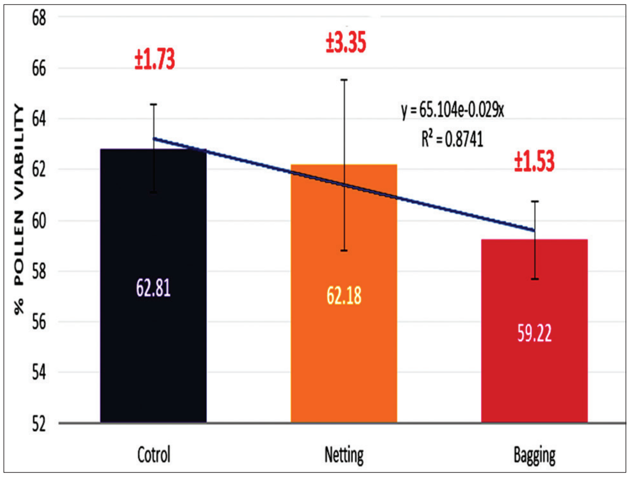

Figure 6: Effect of netting and bagging on pollen viability

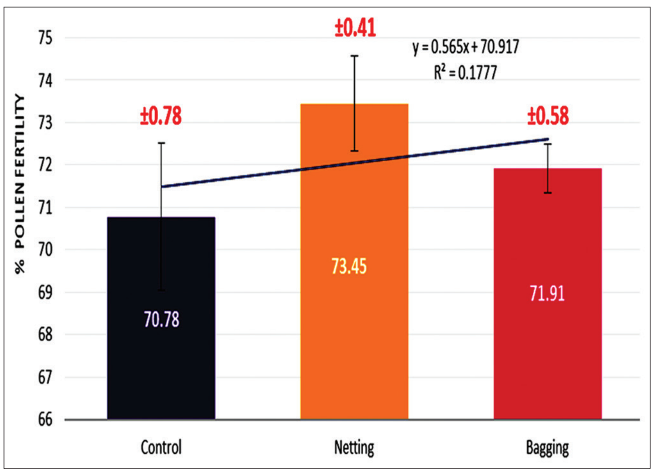

Figure 7: Effect of netting and bagging on pollen fertility

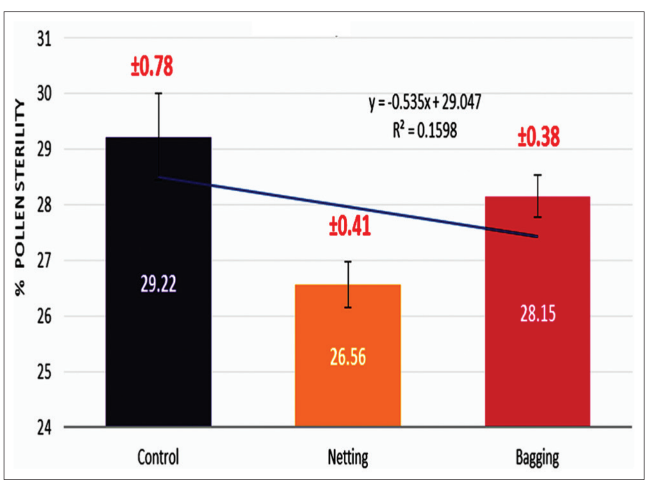

Figure 8: Effect of netting and bagging on pollen sterility

fertilize the ovule in varied conditions. The results highlight the importance of relative floral density within patches of floral resources in shaping outcomes of interactions for pollens and pollinators. Although the total number of flowers in each array varied, relative floral density remained

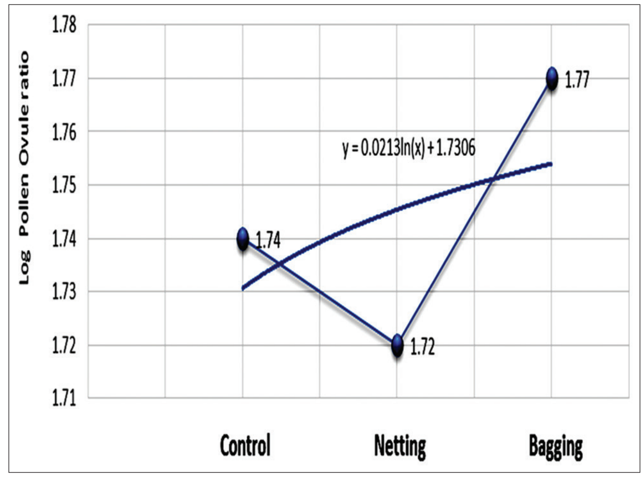

Figure 9: Effect of netting and bagging on log pollen-ovule ratio

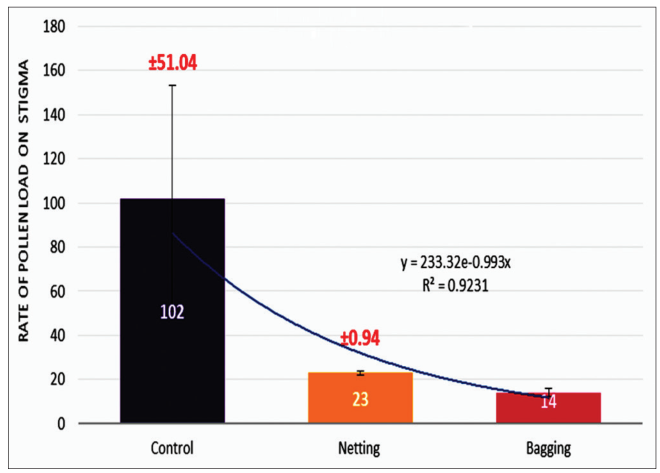

Figure 10: Effect of netting and bagging on pollen load on stigma

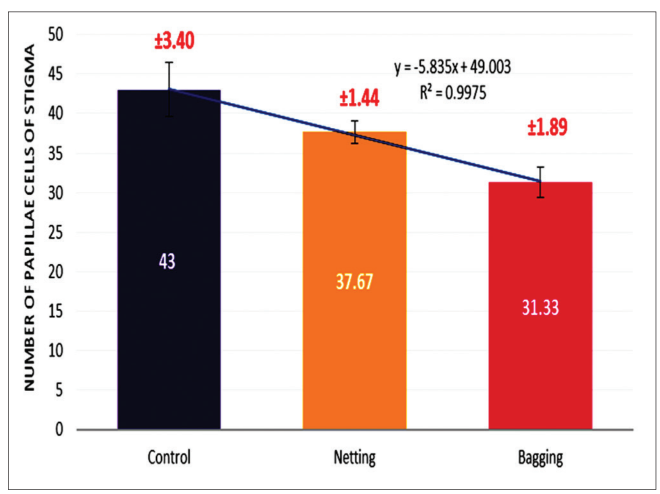

Figure 11: Effect of netting and bagging on number of papillae cells of stigma

constant, and responses of pollen biological features were consistent with the hypotheses about the effects of relative floral densities upon reproductive fitness. While strong interaction effects for pollination are expected for species of

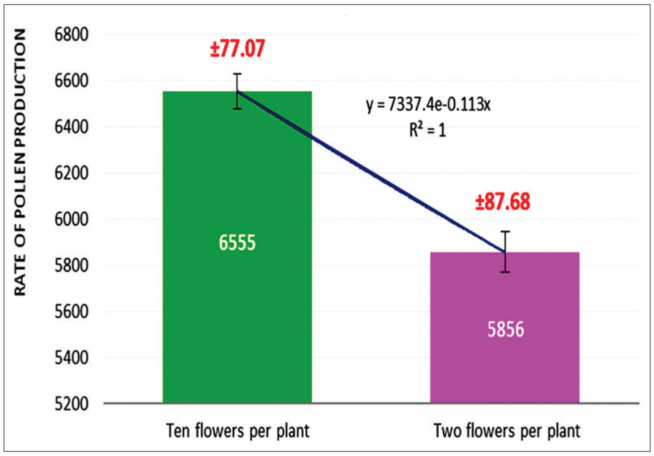

Figure 12: Effect of flower density on pollen production/flower

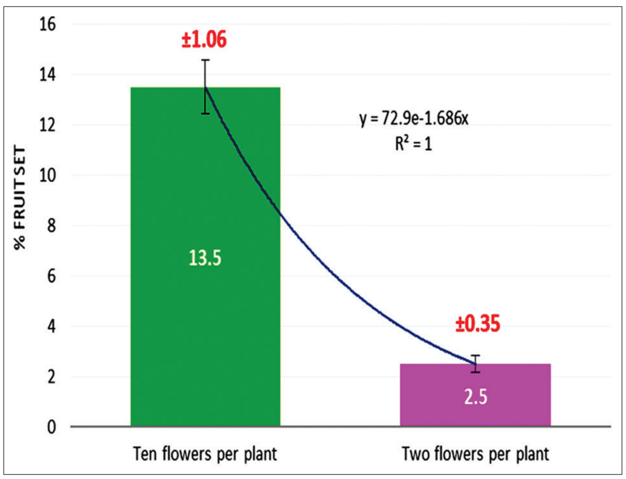

Figure 13: Effect of flower density on fruit set

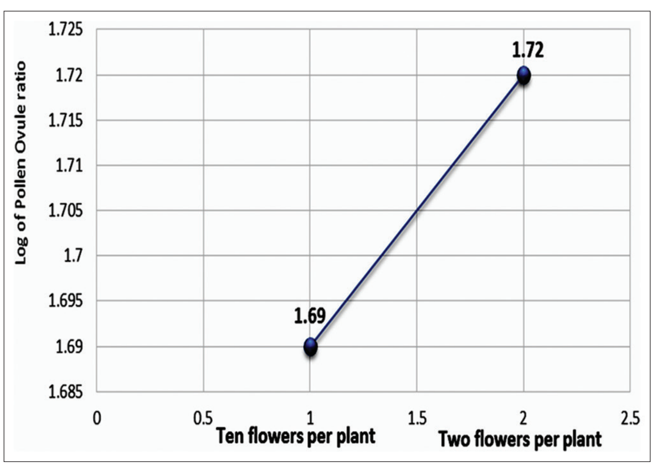

Figure 14: Effect of flower density on log of pollen-ovule ratio

similar growth form and floral morphology, it is found that interaction effects, in this case, facilitation, can also be quite strong for pairs of species with non-similar morphologies (Ghazoul, 2006). In addition to floral morphology, floral reward should also influence the relative floral densities at

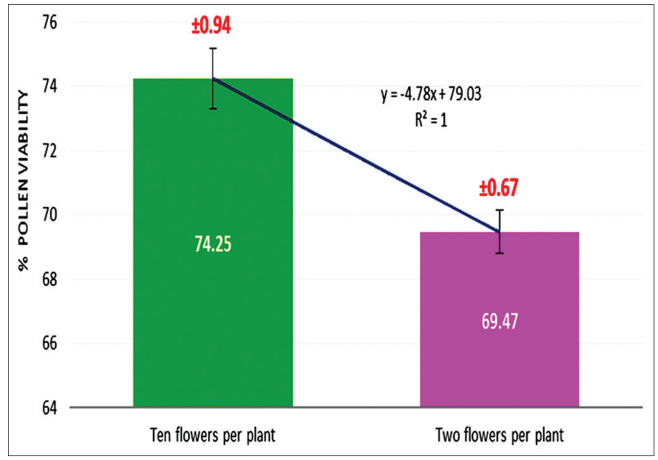

Figure 15: Effect of flower density on pollen viability

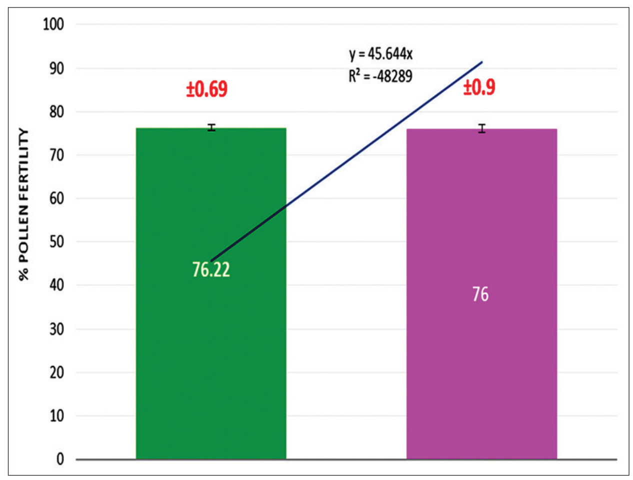

Figure 16: Effect of flower density on pollen fertility

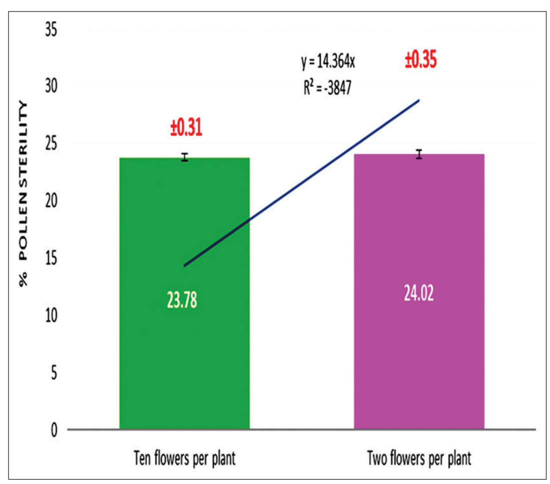

Figure 17: Effect of flower density on pollen sterility

which facilitation and competition for pollination success occur. The results suggest densities are necessary to observe negative effects on *C. gracilis* of the family *Calceolariaceae* pollination. Increased pollen receipt at a low density seems to be a result of a shift in the behavior of individual pollen. The flowers received more than twice as many visits in low density arrays compared to controls, but not significantly more visitors, indicating that visitation rate increased because individual pollinators made more visits. This is in contrast to the more common trend (Feldman *et al.*, 2004; Yang *et al.*, 2011 and Dietzsch *et al.*, 2011). Finally, if flower production is an indicator of maternal resources, it is possible that *C. gracilis* of the family *Calceolariaceae* plants in low-density arrays were unable to take full advantage of increased conspecific pollen deposition, because plants in low-density arrays had significantly fewer flowers than plants in control arrays. Indeed, conspecific pollen deposition was above that required for maximum seed production in *C. gracilis* of the family *Calceolariaceae*, suggesting resource rather than pollen limitation corroborating the views of Mathur and Ram (1986), Greenleaf *et al*. (2007) and Hanoteaux *et al*. (2013). This report of a stigma that does not produce secretions (dry-type stigma) is in conflict with earlier reports that develop a wet-type stigma (Faegri and Pijl, 1981; Bhattacharya and Mandal, 2004). The dry stigma may be best interpreted as convergent with stigmas of other wind-pollinated systems distributed through the angiosperms. While the relative contribution of wind versus insect pollination remains to be determined, there are two overlapping reasons why the non-directional mode of pollen dispersal by air currents may be present in this species. First, pollen load analyses indicate that the nectarless flowers "share" their floral foragers. Cross-pollination is essential for seed set but it will not occur if a bee or fly forages for pollen on a single plant and then switches over to flowers. Second, it blooms in early. Wind pollination might be retained as a "failsafe mechanism" ensuring some level of cross-pollination in this species when the flowers of competing plants or climatic conditions restrict or terminate the movements of insect pollinators. Less fruit production as compared to flowers may be due to delayed receptivity or unavailability of pollinators or pollen competition. Accumulation of somatic mutations in different natural plant population might be a concept to our understanding of the pollen sterility and viability. Although the flowers have viable pollen, self- and cross-pollination with other flowers may results in a significant increase in fertile and more viable pollens which is yet to be studied.

CONCLUSION

It is concluded that the studies on pollen biology of selected species across a range of natural populations that vary in floral density, combined with explicit experimental manipulations of floral density, can provide an important index of the influence of floral density on the sexual fitness of this species. Such studies are particularly important in a modern context, as a wide variety of plant species are becoming increasingly rare as a result of a variety of anthropogenic influences. Although the flowers have viable pollen, self and cross-pollination with other flowers may results in a significant increase in fertile and more viable pollens which is yet to be studied.

ACKNOWLEDGMENTS

The author is thankful to the Officer-in-Charge of Darjeeling Government College for providing necessary facilities to carry out this work and to UGC (ERO) for providing financial support in form of sanctioning the Minor Research Project No. F.PSW-184/15-16 (ERO).

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