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Pollen Limitation in *Eremostachys superba* Royle *ex* Benth. (Labiatae) and its Role in Loss of Seed Production

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ABSTRACT : The critically endangered *Eremostachys superba* Royle *ex* Benth. shows almost negligible sexual reproduction under natural conditions (<= 5%) and survives only vegetatively. Investigations on the role of pollination biology in the loss of sexual reproduction revealed a pollinator limitation in the natural habitat. Increased floral attractiveness was provided to counteract this pollinator limitation syndrome by increasing flower density. Observations were made on insect visits and resultant seed set in the *ex situ* plants and compared with that observed under *in situ* conditions. Infinite pollinators visited *ex situ* flowers but affected only 38% seed set, indicating a reduced fertilization due to geitonogamous pollination. These results suggested that lack of sexual reproduction in *E. superba* was partly due to pollen limitation.

KEY WORDS : ***Eremostachys superba*, Effective pollen flow, Geitonogamy, Pollen limitation.**

INTRODUCTION

The species *Eremostachys superba* Royle *ex* Benth. (Labiatae) was reported as endangered by Jain and Sastry (1984), with a single limited population of *ca* 100 individuals in the Mohand forest area of Dehra Dun. In recent surveys made by the authors in the years 1992 –95 only 33 (±10) extant mature plants could be located and there were no seedlings. The plants survived by means of a thick root stock which generally produced a single (sometimes two) spike in the flowering season. The decline in population size suggested that the reproductive capacity of this species had fallen below its rate of elimination. To find out the causal factors responsible for this decline, studies were undertaken on the reproductive biology during the years 1992 – 96. Possible factors underlying failure of sexual reproduction in the species are outlined in Table 1. It was observed that the species is self-incompatible, with 6.7% geitonogamous and 44.4% xenogamous outcrossing (Rao and Garg, 1994). Pollination biology studies revealed a pollinator limitation in this euphilic species, but the pollinators were scanty. A specialised plant-pollinator co-adaptation existed which ensured successful pollen transfer to the stigma

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(Garg and Rao, 1996). The present investigation was made on the post-pollination events which precede fertilization to study "good - quality" pollen deposition on the stigma, and eventual seed set. The pollinator limitation syndrome was counteracted by increasing floral attractiveness due to increased floral density and by planting the spikes contiguously in the experimental plot. The effect of increasing floral attractiveness was observed in relation to pollination and eventual seed set. The role of phenological pattern in limiting "effective" pollen flow was also considered since both size and population structure of clones interact to modify pollen deposition pattern, effective pollen flow and hence potential seed set (Handel, 1985; Aspinwall and Christian, 1992).

Table 1. Possible causes for the failure of sexual reproduction in insect pollinated *Eremostachys superba* in its natural habitat.

1. The species is self-incompatible, adapted for outcrossing.
2. Increased auto- and geitonogamous pollination due to limited interplant movement.
3. Lack of genotypic diversity due to limited number of plants in single population
4. Pollinator limitation in natural habitat due to scanty flowering spikes.
5. Dispersal of viable seeds, but viability exists only upto one month. Hence, seeds do not germinate in the ensuing rainy season which commences three months later (July).

MATERIALS AND METHODS

As many as eight root stocks of *E. superba* were planted contiguously under *ex situ* conditions (Fig. 1).

Pollen estimation

Pollen analysis was done to estimate pollen production per flower and assess pollen fertility. To estimate pollen quantity one mature but undehisced anther was cut into four equal parts. One part was gently squeezed in 20 drops of glycerine. Pollen content of 10 drops of this glycerine was counted under a compound microscope and then doubled to get the total pollen content per anther. From this the pollen production per flower was calculated (each flower possessed only four anthers). Pollen fertility was determined by dusting fresh pollen in a drop of Alexander's stain on a slide (Alexander, 1980). Sterile (unstained) and fertile (stained) pollen grains were then counted.

Anthesis and stigma changes

Stigma receptivity and anthesis were visually checked using 40x lens. As soon as the flower opened, the receptive surface appeared fresh, pappillose and slightly sticky with diverging arms. By the fourth day of bloom receptivity was completely over. Anther dehiscence was simultaneous to flower opening and stigma receptivity (Table 2).

Fig. 1. *E. superba* under *ex situ* conditions showing simultanteously flowering contigous spikes.

Table 2. Pattern of senescence changes in *E. superba.*

Bud opening	Corolla	Anthers	Stigma
1st day	Fresh, bright yellow, spreading	Dehiscence, pollen release insects visit	Fresh, sticky
2nd day	same as on 1st day	Empty, white, insects visit but abandon	Pale dry
3rd day	Dry and drooping	Withered, brown no insects visit	Withered and necrotic
4-5th day	Withers and corolla tube detatched leaving the persistent calyx	Black,shrunken; falls off with corolla tube	Black, necrotic and persistent

Field observations

Field observations were made on all of the simultaneously blooming flowers. They were counted both *in situ* and *ex situ* in order to assess the floral attractiveness in relation to pollination and pollen flow to the pistil in the year 1995. Pollinators were trapped for the identification of insect and pollen content on their bodies.

Breeding experiments

The following breeding experiments were conducted (Garg and Rao, 1997): A part of the spike with 10 mature buds was bagged using butter paper to check the efficacy of autogamy. Nine flowers were hand pollinated using a brush with the pollen from flowers of the other plant and bagged to observe xenogamous seed output. The remaining 69 flowers in the inflorescence were left undisturbed for assessing the status of natural pollination.

Seed production

Seeds were harvested after 25 days of pollination. Each flower was expected to produce four seeds. From the total number of seeds obtained, the ratio of viable (healthy) and sterile seeds (empty shrivelled up) was calculated.

RESULTS AND DISCUSSION

All eight spikes flowered simultaneously under *ex situ* conditions, whereas *in situ* population had only one or two simultaneously flowering spikes. Pollen production per anther was ample being as high as 33300 −33900 and upto 1,33,800 −1,35,000 per flower. This high pollen production by each flower reflects the probability that a sufficient number of pollen grains will reach a stigma (Cruden, 1977). Pollen fertility was assessed to be 95%. Anther dehiscence and stigma receptivity were almost simultaneous (Table 2). All these factors fulfilled the precondition for pollination and seed set.

Field observations

There was a marked difference in pollination of the species in the year 1995 in the experimental plot. The pollinating insects (*Nomia rustica*) were the same as those in the earlier years. The pollen attached to their body parts was purely of *E. superba*, suggesting an effective pollen transfer to the pistil.

Breeding results

The apparantly viable nutlets were healthier, bigger and dark brown compared to the sterile ones which were empty, shrivelled and weightless. There was only 2.5% seed set from autogamy, 6.7% from geitonogamy and 44.4% seed set from xenogamous operations (Fig. 2). These results indicated a high self-incompatibility with xenogamy being the main mode of sexual reproduction. Xenogamous pollen was thus identified as ~"good quality" pollen for genetic exchange and multiplication in *E. superba*.

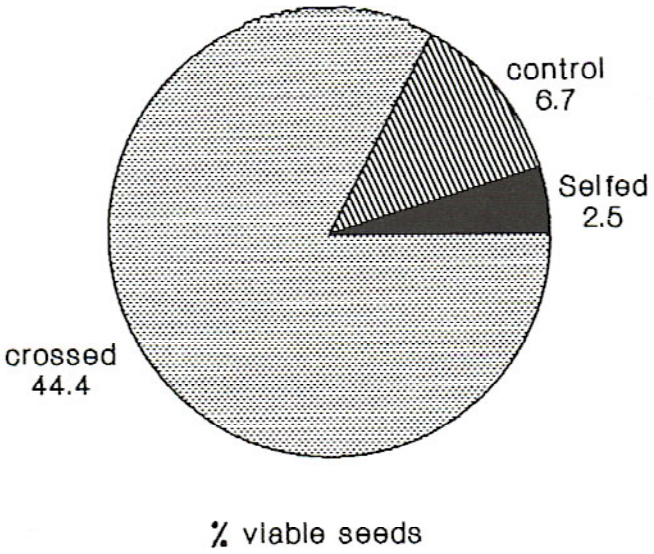

Fig. 2. Breeding results in *E. superba*.

Phenology and pollinator visitation

(i) in natural habitat

Only two simultaneously flowering spikes, one with 65 and the other with 85 flowers, were in bloom (with a total of 30 flowers in bloom) at one time. These spikes were visited by one or two *C. heiroglyphica* bees. These pollinators generally confined their flights to the same spike; there was seldom crossing from one spike to the other as the distance between the two flowering spikes was about 15 ft. These flowers when left for seed set through natual pollination yielded only 5 viable seeds against the expected 120 seeds. Hence there was only <5% seed set under *in situ* conditions through natural pollination. Geitonogamy and selfing were the main modes of pollen flow. Genetic mixing and seed production was therefore curtailed in this self-incompatible species to as low as 5% under *in situ* conditions. As suggested by Bawa and Opler (1975), the limited interplant movements of small pollinators result in high levels of selfing in harmaphrodites.

(ii) in the experimental garden

The eight rootstocks produced 10 contiguous spikes (Fig. 1). All the spikes flowered almost simultaneously (with difference of one or two days in flower initiation). The smallest spike produced 45 flowers while the largest one had 120 flowers arranged in 12 verticillasters. About 300-350 flowers were together in bloom under *ex situ* conditions in contrast to the 30 flowers of the natural habitat. This number, although sufficient to attract a wide spectrum of pollinators, was visited specifically by *Nomia rustica* bees, exemplifying the euphilic nature of the plant species. It was observed that –

1. Hundreds of bees visited the flowers generally around 8:00 a.m. and worked until 3:00 - 4:00 p.m. The numbers of insects were so large that they could not be counted. No insect other than *N. rustica* visited the flowers. Richards (1986) reported that the pollination dynamics of a plant can change as a consequence of its growth pattern. The simultaneous and contiguous flowering resulted in a large number of densely blooming flowers which attracted more pollinators. This was in contrast to the *in situ* plants with only 30 flowers, visited by just one or two pollinators at a time. Further, the pollinators body laden with pure pollen of *E. superba* exemplified an efficient transfer of the male reproductive unit to its female counterpart, a necessary precondition for pollination success in the event of fertilization and seed set.
2. There was a swift and random movement of the bees within and in between different spikes. Hence there was a random exchange of genetic material (both auto- as well as allogamous). Assessment of pollen as "high" and "low" quality pollen was made on the basis of studies of Garg and Rao (1997), which revealed a high (44.4%) seed set by xenogamous pollination, 6.7% by geitonogamous and 2.5% by autogamous pollination (Fig. 2).

Seed set

Only 980 viable seeds were produced against the expected 2560 sterile seeds i.e. there was only 38% seed production indicating 62% failure in seed set.

The results suggested that in spite of effective transfer of the pollen to the pistil there was still not enough seed production. This is a true representation of inefficient sexual reproduction, which in turn implies a discrepancy in the post-pollination events. However, the lack of seed production is not controlled by any single factor. Many factors contribute toward this loss. Pollen limitation on the stigma is a frequently cited factor in low fruit and seed set in both insect and wind-pollinated plants (Biezychudek, 1981; Willson and Burley, 1983; Hainsworth *et al*, 1985; Ayre and Whelan, 1989). It includes pollen quantity or viability (Fox, 1992) and compatability of donor or recipient plants (Lovett-Doust *et al*, 1986). The limitation of compatible (or useful) pollen on stigmatic surface is one of the most plausible factors which upset the reproductive cycle and is responsible for the low seed set. A high pollen density of low quality pollen (geitonogamous and autogamous) on the stigma blocks space on the stigmatic surface for compatible (xenogamous) pollen deposition and serves as the primary factor responsible for pollen competition, and germination of the "high quality" (xenogamous) pollen. This is further explained as follows:

Geitonogamy is a non-adaptive feature of outcrossing as it does not offer any reproductive assurance and is likely to result in decreased male contribution to outcrossing in self-incompatible species. Increasing floral attractiveness associated with increasing the number of flowers on a plant may result in prolonged pollinator visitation and increased frequency of geitonogamy and hence selfing (Charlesworth and Charlesworth, 1987). In the experimental plants the pollinators worked exhaustively and in random ways on the flowers for hours. This resulted in a dense pollen deposition on the stigma surface, with the surface covered with all types of pollen (auto-, geitono-, xenogamous). Further, the species *E. superba* has been shown to be self-incompatible (Rao and Garg, 1994) with very low rate of geitonogamy (6.8%). Since xenogamy is the only mean of sexual reproduction in this species, only xenogamous pollen is the high quality compatible pollen, while the rest are considered as incompatible/ low quality pollen, which block the stigma surface and result in compatible pollen limitation.

Germination of some of the incompatible pollen on stigma blocks the space in the stylar canal for the compatible pollen tubes, enhancing the level of pollen competition. This situation persists until the act of fertilization. Hence, very few compatible male gametes are able to reach and fertilize the female gametes, creating pollen limitation.

CONCLUSION

Clonal growth pattern coupled with self-incompatibility increases the probability of geitonogamy and hence ineffectual pollen deposition on the stigma that in turn decreases seed set (Handel, 1985).

Although *E. superba* is pollinated by legitimate pollinators (*N. rustica*) which appeared to be a specialist by their ethodynamic, oligolectic and sternotribic approach toward pollination, still, they are likely to transfer low quality, incompatible pollen (auto-, and geitonogamous) to the stigma and be ineffective in achieving cross-fertilization. Incompatible pollen blocking the stigmatic surface interferes with compatible pollen deposition and creates competition for the compatible pollen in fertilization and seed set. Hence, pollen limitation was partly observed to be the most plausible explanation for low seed set in *E. superba* in the presence of inumerable legitimate pollinators.

To conclude, the lack of seed production is not controlled by a single factor. They are: 1. Malfunctioning in pollination biology is examined as a potential explanation for reproductive failure. Few spikes on the flower at a time, hence is a pollinator limitation; 2. Species is self-incompatible. Crossing is scanty due to pollinator limitation; 3. Even when there is plentiful pollination, a pollen competition occurs on stigma surface for cross-pollen to effect in seed set; and 4. The few seeds reared by hand pollination also failed to germinate under natural condition as viability is lost in one month.

In addition to pollen dynamics, other factors restricting development of fertilized embryos could also contribute to the lack of viable nutlets in the species (as outlined in table 1) which includes physiological factors related to reduced rates of photosynthesis, *i. e* allocation of vegetative growth, and lack of sufficient summer heat units to mature the embryos (Sarvas, 1962; Elliott, 1979; Richards, 1986; Kullman, 1987).

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LITERATURE CITED

Alexander, M. P. 1980. A versatile stain for pollen, fungi, yeast and bacteria. Stain Technology **55** : 13-18.

Aspinwall, N. and T. Christian. 1992. Pollination biology, seed production, and population structure in Queen-of-the-praire, Filipendula rubra (Rosaceae) at Botkin Fen, Missouri. Amer. J. Bot. **79** : 488-494.

Ayre, D. J. and R. J. Whelan. 1989. Factors controlling fruit set in hermaphroditic plants : studies with the Australian Proteaceae. Trends in Ecology and Evolution **4** : 267-272.

Bawa, K. S. and P. A. Opler. 1975. Dioecism in tropical forest trees. Evolution **29** : 167 - 179.

Biezychudek, P. 1981. Pollinator limitation of plant reproductive effort. American Naturalist **117** : 838 - 840.

Charlesworth, D and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. **18**: 237-268.

Cruden, R. W. 1977. Pollen-ovule ratios : a conservative indicator of breeding systems in flowering plants. Evolution **31** : 32-46.

Elliott, D. L. 1979. The current regenerative capacity of the northern Canadian trees, Keewatin, N.W.T., Canada, some preliminary observations. Arctic and Alpine Research **11**: 243-251.

Fox, J. F. 1992. Pollen limitation of reproductive effort in willows. Oecologia **90** : 283 - 287.

Garg, A. and R. R. Rao. 1996. Pollination ecology of the endangered Eremostachys superba Royle ex Benth. (Labiatae). Taiwania **41** : 309-321.

Garg, A. and R. R. Rao. 1997. Observations on the Reproductive biology of endangered Eremostachys superba (Labiatae). Proc. Indian natn. Sci. Acad. **B63** : 79-88.

Hainsworth, F. R., L. L. Wolf and T. Mercier. 1985. Pollen limitation in a monocarpic species, Ipomopsis aggregata. of Ecol. **73** : 263 - 270.

Handel, S. N. 1985. The intrusion of clonal growth patterns on plant breeding systems. Amer. Naturalist **125** : 367-384.

Jain, S. K. and A. R. K. Sastry. 1984. The Indian Plant Red Data Book - I. Botanical survey of India, Howrah.

Kullman, L. 1987. Long-term dynamics of high altitude populations of Pinus sylvestris in the Swedish Scandes. J. of Biogeography **14** : 1-8.

Lovett-Doust L., J. Lovett-Doust and K. Turi. 1986. Fecundity and size relationships in Jack-in-the-Pulpit, Arisaema triphyllum (Araceae) Amer. J. Bot. **73** : 489-494.

Rao, R. R. and A. Garg. 1994. Can Eremostachys superba be saved from extinction ? Current Science **67** : 80-81.

Richards, A. J. 1986. Plant breeding systems. Allen and Unwin, London.

Sarvas, R. 1962. Investigations on the flowering and seed crop of Pinus sylvestris. Comm. of the Institut of Forestry Fennica **53** : 1-198.

Willson, M. F. and N. Burley. 1983. Mate choice in plants. Princeton University Press. Princeton, NJ.

印度沙穗的花粉量限制在降低種子產量中所扮演的角色

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摘　要

瀕臨絕種的印度沙穗(唇形科)在自然環境下有性繁殖低於 5%，而只以無性繁殖得以存續。經授粉生物學研究，得知自然環境下因授粉者限制了有性繁殖，故增加花的數量可補強授粉者之限制。觀察在原地及移地下比較昆蟲造訪及其結實，發現雖然移地下使昆蟲不斷造訪，卻只結實 38%，表示自株授粉會減低受妊。由結果表示印度沙穗欠缺有性繁殖乃部份受到花粉量之限制。

關鍵詞：印度沙穗、有效花粉量、自株授粉、花粉量限制。

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