

The Part Taken by Cleistogamy in *Narthecium ossifragum* Reproductive Strategy

ANNE-LAURE JACQUEMART*) and JACQUES R. DE SLOOVER

Laboratoire d'Écologie et de Biogéographie, Louvain-la-Neuve, Belgium

Summary

The floral biology, breeding system and pollination ecology of *Narthecium ossifragum* (Liliaceae) were analysed in the high Ardennes, Belgium, during the summer 1986. A new kind of pollination for this species was noticed: some flower buds don't open but set seeds. Observations substantiate the claim of self-fertilization which is connected to pseudocleistogamy. A probable cause for this bud pollination is drought. Nevertheless, its efficiency and frequency are very low.

Key words: pollination, floral biology, pseudocleistogamy, *Narthecium ossifragum*.

1. Introduction

A genetic model has been proposed by SCHEMSKE & LANDE (1985) which predicts that in many situations both extremes of predominant outcrossing and predominant self-fertilization represent alternative stable states of the mating system. On the contrary LLOYD (1979) stated that "... angiosperms show a wide range of intermediate frequencies of self-fertilization ..." so that LORD (1981) could conclude that an intermediate frequency of selfing would be expected in many populations.

The present study attempts to do an appraisal of the relative significance of pollination outcrossing or selfing in *Narthecium ossifragum*. The flowers of that species are conspicuous, homogamous, unspecialized and offer considerable pollen rewards. They thus probably attract a great diversity of flower visitors (SUMMERFIELD 1974) and are a good example of those "promiscuous" plants (LINDSEY 1984). Assuming insect pollination takes a leading part, what about the other pollination systems?

HAGERUP (1950) claimed that rain pollination is predominant in the Faroe Islands. His conclusions are widely admitted (STEBBINS 1957; MOORE & LEWIS 1965; SUMMERFIELD 1974; PERCIVAL 1979; SCHOEN & LLOYD 1984). DAUMANN (1970) alone doesn't agree and takes into account the injuries done to the stigmas and to the pollen by the rain. We thus attempted to appraise the relative part of each of these pollination systems. Furthermore, during summer 1986, we came accross floral buds which never opened but set seeds. We thus describe that newly observed bud pollination and discuss the nature, occurrence, efficiency, and origin of this likely cleistogamy.

*) Correspondant author.

2. Materials and Methods

2.1. Study sites: From July 16 to 29, 1986, experiments were conducted intensively at two sites located in the Plateau des Tailles area near the place called Baraque de Fraiture (5°44'22" Long.E., 50°15'00" Lat.N.), in High Ardennes (alt. 652 m), Belgium. Observations were made in a *Narthecium* dominated peat bog and in a peaty heath.

2.2. Experiments: Data on the breeding system were obtained by controlled hand pollinations and bagging experiments. Flowers were protected from insect visitors by the use of fine mesh nylon bags that enclose the entire inflorescence (21 inflorescences per treatment and site). Five experimental categories were established to check the part taken by rain pollination (artificial rain), by selfing (hand pollination) or by cross-pollination:

Treatment 1: open pollinated controls

Treatment 2: flowers open pollinated but daily sprayed with water (artificial rain pollination)

Treatment 3: bagged flowers hand pollinated

Treatment 4: bagged flowers daily sprayed with water

Treatment 5: bagged flowers unmanipulated designed as controls.

A census was taken of all flower visitors which were timed and classified as much as possible in the field.

2.3. Further observations: During field studies, an increasing number of withered racemes was noted. In other respects, some flowers didn't show anthesis, flower buds remaining in an arrested stage during the whole flowering period. Some of those closed buds were fixed in FAA (1:1:9 formalin-acetic acid-alcohol), embedded in paraffin and sectioned at 10 µm. Serial sections were mounted, stained with aniline blue and scrutinized under fluorescent lighting microscope. The stain colours callose specifically which is present i.e. in exine and pollen tubes. A second sample of those arrested buds was tagged and followed till fruit set. Seeds were counted and the results compared with those achieved by other treatments.

Germination tests were conducted according to SUMMERFIELD's methods (1973). Statistical analysis were made using S.A.S. programs (HELVIG & COUNCIL 1979).

3. Results

3.1. Cross pollination

Results of the field experiments point out the outstanding importance of cross pollination (Table 1: Treatment 1). *Narthecium* attracts a great diversity of floral visitors, especially *Bombus* sp. and a number of Syrphids including *Eristalis tenax*, *Episyrphus ribesii*, *Episyrphus balteatus* . . . Of the 542 individual insects observed on *Narthecium* in the Plateau des Tailles area 93% were Syrphids and 6% were *Bombus*. Nevertheless, if *Bombus* pollinator's activity was more restricted by cold or rainy weather, it seemed the most efficient in foraging. Measurements of pollinator effectiveness should be reinvestigated.

3.2. Bud pollination

When the fruit set in unopening flower buds is compared with that in opening flowers, it is clear that bud fertilization does occur but that it is not as efficient as cross pollination. As a matter of fact, "cleistogamous" flowers set 15.8 seeds per capsule against 56.7 in naturally pollinated flowers and mature 62.5 against 96.7% of their buds (Table 1). In serial

Table 1. Seeds set per capsule and fruit set in *Nartheicum ossifragum* at the Plateau des Tailles. The stars mentioned at the right of the numbers indicate no significant difference according to the results of Neuwman's test and Scheffe's study (seeds set per fruit). Results of fruit set and germination rate show no significant difference.

Treatments	Criterion			
	Seeds set per fruit ($\bar{x}$)	No. caps (%)	Fruit set (%)	Germination rate (%)
Treatment 1 Naturally pollinated	56.7	222	96.7*	26.8*
Treatment 2 Open and rain pollinated	49.5	203	95.0*	7.6*
Treatment 3 Hand (self) pollinated	38.0*	194	88.5*	12.2*
"Cleistogamy"	15.8*	40	62.5*	4.4*
Treatment 4 Bagged unmanipulated	13.1*	207	67.8*	6.0*
Treatment 5 Bagged and rain pollinated	8.5*	204	65.4*	26.5*

sections, numerous fluorescing plots appeared clearly below the stigma under fluorescent lighting microscope. Every one of the sectioned styles in unopened buds showed pollen tube callose. The anthers were dehiscent and pollen grains released. These pollens were on stigmatic papillae and had germinated into the style. Bud pollination is thus effective. We didn't either note any significant difference between both sites. The ratio of this bud pollination could be estimated as follows: the average number of flowers per inflorescence is 19.3, about 80 percent of racemes have only one (Table 1) unopening flower bud and 1/5 or 1/10 of the raceme displays those "cleistogamous" flowers. So, unopening buds account roughly for 0.6 to 1% of the studied populations. Most of them occurred in the lower part of the racemes: first or second positions at respectively 34.3 and 28.8%. Seeds appeared only viables, failure in germination tests doesn't enable greater debates.

3.3. Rain pollination

The percentage of fruit set and mean seeds per fruit decreased in flowers receiving artificial rain (Table 1: Treatment 1 versus Treatment 2 and Treatment 4 versus Treatment 5). The reduced seed set from rain pollination may have resulted from pejorative effect on stigmas and pollens as DAUMANN (1970) stated. But the detrimental effect could be due to the artificial rain (sprayed water): the water didn't stay in the flower cups for a long time and/or didn't fill up the flowers as HAGERUP (1950) observed.

4. Discussion

4.1. Cleistogamy refers to the production of flowers that do not open and do self in the bud. The term is more often used to describe species which produce both open (chasmogams-CH) and closed (cleistogams-CL) flowers which never achieve anthesis but set good seed.

Observations of pollen tubes growing in the styles of unopening flowers and results of fruit set, even if restricted, substantiate the claim of self-fertilization in some *Narthecium* buds. Cleistogamy (or induced selfing according to SCHOEN 1984) thus occurred in *Narthecium* populations during the summer 1986. This is the first report of that process in *Narthecium*.

4.2. LORD (1981) proposed four categories in order to enlighten the differences between the many cleistogamous species. In *Narthecium* no morphological difference occurs between CL and CH flowers except a lack of growth and of anthesis in CL flowers. This case is referred to "Pseudocleistogamy". It's different from "true" cleistogamy in which CL flowers are modified forms of the CH flowers mainly characterized by reductions in stamen size and/or number and corolla size. Such reductions are due to more than a mere lack of growth. The pseudocleistogamous flowers usually have the ability to self in the bud so there may be some subtle physiological modification in the closed flowers (LORD 1981). The observed bud pollination in *Narthecium* is here connected to that pseudocleistogamy.

4.3. A genetic basis has been suggested for cleistogamy in a number of species (BATES & HENSON 1955; LORD 1981) but in any case, environmental factors influence the production of CL and CH floral forms. The controversy over whether or not cleistogamy was only induced by the environment probably arose from the fact that "cleistogamy" referred to a range of phenomena. Usually poor growth conditions are inducing a predominance of CL flowers.

According to several authors (UPHOF 1938; LORD 1981) pseudocleistogamy is largely influenced by environmental factors such as drought. For example, BROWN (1952) experimenting on *Stipa leucotricha* concluded that when available soil water is reduced, percent of CL flowers increases significantly.

In the case of *Narthecium* some observations make a point concerning the influence of drought in pseudocleistogamy. First, during the summer 1986 weather was relatively sunny and dry (Table 2). The water table lowered and some inflorescences withered. So a relative drought could influence the formation of PCL flowers. Moreover, the same experiments were carried out during the summer 1987 and during the flowering period which was very rainy, no one inflorescence has been withering (Table 2). All the flowers showed anthesis and cleistogamous flowers didn't appear: it's thus conceivable that the observed pseudocleistogamy could be due to drought.

Table 2. Ten-days mean rain data (mm) at 3 localities in High Ardennes during July 1986 and July 1987 (no data were available from Baraque de Fraiture).

Periods	Libramont		Saint-Vith		Botrange	
	1986	1987	1986	1987	1986	1987
01-10.07	4.67	0.06	3.82	1.60	6.12	0.02
11-20.07	0.69	9.48	0.35	5.30	0.53	5.95
21-31.07	0.84	5.24	2.27	5.68	1.99	9.12

Furthermore, even though pseudocleistogamy was substantiated in *Nartheicum* its efficiency and frequency are very low. More successful CL seed production was reported in several cleistogamous plants (e.g. SCHEMSKE 1978; LORD 1981; SCHOEN 1984). Greater maternal expenditure and fertilization success in the CL component are two theoretical expectations for the evolution of cleistogamy as proposed by SCHOEN & LLOYD (1984). Ecological and genetical differences between the progeny are obvious. A "true" CL flower (modified) shows at least fruit set as well as CH. Nevertheless, there can be differences in dispersion, germination or cost. The same doesn't hold good for *Nartheicum* which ripened few seeds with the same (?) germinating capacity than CH flowers. It is not known whether those seeds are effectively spread later and if they meet other seedling establishment conditions. Pseudocleistogamy in *Nartheicum* could thus meet some temporary unfavourable environmental conditions. On this point further observations are still required.

5. Conclusions

Some original observations on pseudocleistogamy in *Nartheicum ossifragum* (L.) HUDS. in the high Ardennes are gained and an interpretation of the floral biology as a whole is given.

Apparently, under favourable conditions, *Nartheicum ossifragum* is adapted to cross pollination by insects. We conclude with SUMMERFIELD (1974) that local populations of "generalist" insects are the major pollinators.

Moreover, *Nartheicum ossifragum* under unfavourable dry environmental conditions acquires a fertility insurance through different self-fertilizations such as bud pollination and/or pseudocleistogamy (e.g. selfing without anthesis but structurally unchanged CL flowers). As STEBBINS (1957) stated, self-fertilization may result from temporary or local environmental changes and thus doesn't show constancy. Automatic self pollination (affected by the juxtaposition of the mature anthers and stigma) and/or preanthesis cleistogamy (bud pollination followed by anthesis) also occurred in a few cases. Ombrogamy wasn't clearly substantiated.

Nartheicum is thus supplied with a "mixed" breeding system.

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Authors' address: ANNE-LAURE JACQUEMART and JACQUES R. DE SLOOVER, Laboratoire d'Écologie et de Biogéographie, 5, Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium