

Causes of Fruit Set Variation in *Polygonatum odoratum* (Liliaceae)

J. Guitián¹, P. Guitián¹, and M. Medrano^{1,2}

¹Laboratorio de Botánica, Departamento de Biología Vegetal, Universidad de Santiago, Santiago de Compostela, Spain

²Current address: Dp. Biología Evolutiva, Estación Biológica de Doñana, C.S.I.C., Sevilla, Spain

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Abstract: In *Polygonatum odoratum* (Liliaceae), flowers located distally within the flowering shoot typically exhibit lower fruit set than flowers located proximally. In this study, experiments were performed to investigate whether the lower fruit set of distal flowers is due to pollen limitation, resource competition or “architectural” effects. We found that (1) fruit set and mean number of ovules per flower decline within the flowering shoot from proximal to distal position; (2) variations in the amount of pollen received do not explain the observed differences in fruit set; (3) the fruit set of the most distal flowers was significantly increased by removal of other flower buds in the flowering shoot just before flower opening, indicating that fruit set in these flowers is resource-limited; and (4) removal of proximal flowers increased the fruit set of later flowers to levels similar to those of early flowers of control shoots, arguing against the architectural effects hypothesis.

Key words: *Polygonatum odoratum*, pollen limitation, resource competition, architectural effects, fruit set.

Introduction

Lloyd (1980^[19]) has postulated that, within a given reproductive season, maternal investment can be adjusted at any of three consecutive stages: flower production, ovary development and fruit maturation. In accordance with this, the commonly observed spatial (i.e., position-related) and within-season temporal variations in number and/or size of reproductive structures may be associated with successive adjustment in maternal investment.

Numerous studies have investigated relationships between reproductive success and flower position (proximal or distal) and/or time of opening (early or late) (for reviews see Stephenson, 1981^[26]; Lee, 1988^[18]), both in entire plants or on branches with multiple inflorescences (see Diggle, 1995^[7]), and within inflorescences (Diggle, 1995^[7]; Brunet, 1996^[4]; Medrano et al., 2000^[20]; references cited in this studies). For example, in species with acropetal inflorescence development, the probability

of fruit set and/or the number of seeds per flower is often lower for distal/late-opening flowers than for proximal/early-opening flowers (Solomon, 1988^[24]; Herrera, 1991^[15]; Ehrlén, 1992^[9], 1993^[10]; Karoly, 1992^[17]; Guitián, 1994^[13]; Guitián and Navarro, 1996^[14]; Navarro, 1996^[22]; Ashman and Hitchens, 2000^[11]).

Various nonexclusive hypotheses have been put forward to explain the proximate causes (*sensu* Stephenson, 1981^[26]) responsible for such patterns of within-inflorescence variation in female reproductive success. (1) The “non-uniform pollination hypothesis” (Thomson, 1985^[29]; Lee, 1988^[18]; Thomson, 1989^[30]; Berry and Calvo, 1991^[3]; Goldingay and Whelan, 1993^[11]; Brunet and Charlesworth, 1995^[5]) postulates that the observed patterns may be attributable to variation in pollen receipt over the inflorescence flowering period. Specifically, the relatively low fruit or seed sets of certain flowers on the inflorescence may be attributable to insufficient quantity or quality of pollen (Lee, 1988^[18]). (2) The “resource competition hypothesis” (Stephenson, 1981^[26]; Bawa and Webb, 1984^[2]; Nakamura, 1986^[21]; Lee, 1988^[18]; Thomson, 1989^[30]; Guitián, 1994^[13]) postulates that the observed patterns are attributable to competition among the ovaries of an inflorescence for a limited amount of resources; fruits initiated early and/or located close to the source of nutrients sequester more resources than fruits initiated later and/or located more distally (Lee, 1988^[18]). (3) Diggle (1995^[7]) has used the term “architectural effects”, and Wolfe (1992^[32]) the analogous term “developmental constraints”, to describe morphological variations which are an unavoidable consequence of the development and architecture of the inflorescence, and which are thus not so susceptible to adjustment. The architectural effects hypothesis (Wyatt, 1982^[33]; Lee, 1988^[18]; Thomson, 1989^[30]; Wolfe, 1992^[32]; Diggle, 1995^[7], 1997^[8]) postulates that the observed patterns are attributable to intrinsic (“architectural”) limitations on the reproductive success of flowers in different positions within the inflorescence (Diggle, 1995^[7], and references therein). This hypothesis is based on studies in which the fruit set of late-opening flowers is not affected by the removal of early-opening flowers or the prevention of their fruiting (Sutherland, 1987^[28]; Berry and Calvo, 1991^[3]).

In the work reported herein, we investigated the importance of the different types of effects in the flowering shoots of the clonal plant *Polygonatum odoratum* (Liliaceae).

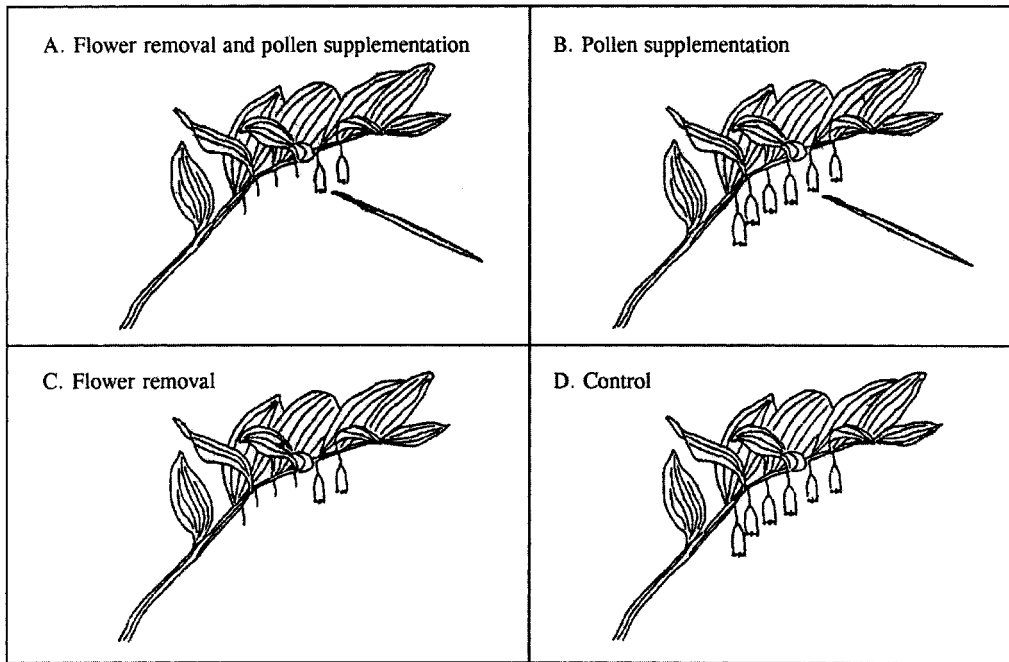


Fig. 1 Habit of *Polygonatum odoratum*, and diagrammatic representation of the experimental treatments applied in the present study.

Having confirmed that both the number of ovules and the probability of producing fruit vary depending on a flower's position within the flowering shoot (see results), we set out to answer the following questions: (1) are the variations in fruit set dependent on the amount of pollen arriving at the flowers?, and (2) to what extent do resource availability and/or architectural effects influence the female reproductive success of flowers at different positions within the flowering shoot?

Polygonatum odoratum was used for two main reasons. First, it has flowering shoots showing acropetal development, with a flowering sequence that allows elimination of the most proximal flowers before opening of the most distal flowers. Second, fruit set varies depending on flower position.

Materials and Methods

Study species and area

Polygonatum odoratum (Miller) Druce (Liliaceae) is a clonal rhizomatous geophyte with shoots of up to 50 cm in height, and with very short-stalked leaves arising from the stem. The shoots arise from rhizome buds. Leaves develop acropetally, and the shoot must reach a certain size and bear a certain number of leaves before becoming a flowering shoot. The mean number of cauline leaves on non-flowering shoots was 5.6 ± 1.2 (range 3–7), versus 10.2 ± 1.0 (range 8–12) on shoots which flowered. These means differ significantly ($t = 16.1$, $p < 0.0001$). Considering all flowering shoots, there was a strongly significant positive correlation between the number of leaves and number of flowers ($r = 0.88$, $p < 0.0001$). The mean number of flowers produced per flowering shoot is 6.8 ± 2.3 (range 4–11). The white tubular flowers develop acropetally (proximal to distal) in cymes of 1 or 2 along the flowering shoot (see Fig. 1). *Polygonatum odoratum* does not show spontaneous autogamy (i.e., it needs pollinator visits for fruit

production). Pollination is largely by bumblebees (*Bombus terrestris* and *B. hortorum*). The fruit is a black berry of about 1 cm in diameter. In the study population, flowering commenced on May 15 and lasted about 30 days. Fruits first appeared on about June 15 and matured in August/September. This species is widely distributed in Europe and also occurs in Morocco. It occurs on shady sites on a variety of substrates in both deciduous and evergreen woodlands.

The study was carried out in the Sierra del Caurel (Lugo Province, northwest Spain), a mountainous area forming the westernmost extension of the Cordillera Cantábrica. The study population comprises several hundred plants located in a wooded area known as Devesa da Rogueira, at 1300 m asl. The dominant tree species are *Quercus robur*, *Betula alba* and *Sorbus aucuparia*.

Experimental design

Among-flower variation. In mid-May 1996, we randomly selected 20 flowering shoots used to characterize within-shoot variation in fruit set in the study population. Another 30 flowering shoots were used to investigate within-shoot variation in ovule number between the most proximal flower and the most distal flower; ovaries were removed for counting of ovules under a stereomicroscope. Five flowering shoots were excluded from the analysis because the distal flower did not show a well-developed style and/or stigma.

Intra-shoot resource competition. Another 32 flowering shoots were randomly assigned to one of the following treatments ($n = 8$ per treatment) (Fig. 1). A. Flower removal and pollen supplementation. The shoots were subjected to flower removal (removal of all but the two most distal flowers, just before opening, while the remaining flowers received supplementary pollination). B. Pollen supplementation. The shoots were not

subjected to flower removal, and all flowers received supplementary pollination. C. Flower removal. The shoots were subjected to flower removal, but the remaining flowers did not receive supplementary pollination. D. Control. The shoots were not subjected to flower removal, and did not receive supplementary pollination. In the flower removal treatments between 1/3 and 1/4 of the flowers of each shoot were eliminated. All flowers included in this experiment showed well-developed style and stigma. Supplementary pollination was performed over a 15-day period, three times per flower on consecutive days, with a mixture of pollen from nearby plants. In total, 112 flowers were pollinated.

During August, all fruits were collected and their dry weights determined in the laboratory, after oven-drying to constant weight at 45 °C. Fruit sets were calculated for all plants in these experiments.

Data analysis

Mean numbers of ovules per flower were compared between distal and proximal flowers using Student's *t* test. Fruit set was compared between proximal and distal flowers using *G* tests. In analyses of fruit set, to allow consideration of flowering shoots with different numbers of flowers, flower position was in all cases defined as "proximal" (the earliest third to open), "intermediate" (the middle third), or "distal" (the last third). For example, flower number 3 in an 11-flower shoot was classified as "proximal" and flower number 4 as "intermediate". The effects of flower position on fruit set were analysed with the Friedman test (Zar, 1996^[34]).

The effects of the different treatments (A–D) on the possibility that a distal flower set fruit were analyzed by logistic regression using the CATMOD procedure contained in the statistics package SAS (release 6.12, SAS Institute, 1996^[23]). This technique, like other logistic regression methods, constructs a log-linear model allowing prediction of the probability of a given outcome (from among two or more possible outcomes), given the values of one or more independent variables. In the present case, each flower was treated as an event with two possible outcomes ("set fruit" or "not set fruit"), and the independent variables were "flower removal" and "pollen supplementation". The effects of the different experimental treatments (flower removal and/or pollen supplementation) on mean single fruit dry weights were investigated by two-way analysis of variance (type III sum of squares). In the text, means are cited \pm standard deviations.

Results

Among flower variation. Mean fruit set per shoot (% flowers setting fruit) was $72.2 \pm 20.9\%$ (range 25–100%). Fruit set varied significantly with within-shoot position (Friedman $\chi^2 = 13.6$, *df* = 19, *p* = 0.001), the mean values ranging from $88.6 \pm 38.9\%$ for proximal flowers to $51.8 \pm 24.4\%$ for distal flowers.

Proximal flowers had significantly more ovules than distal flowers ($x_{\text{proximal}} = 14.4 \pm 2.6$, [range 9–19]; $x_{\text{distal}} = 12.4 \pm 2.3$, [range 5–18]; *t* = 4.3, *df* = 24, *p* = 0.0001).

Table 1 Results of logistic regression analysis to investigate the effects of flower removal and supplementary pollination on the probability (*p*) that a distal flower of *Polygonatum odoratum* will set fruit

Source of variation	Degrees of freedom	Chi square	<i>p</i>
Intercept	1	0.46	0.496
Flower removal (R)	1	5.73	0.017
Supplementary pollination (S)	1	0.06	0.803
R * S	1	0.98	0.323

Intra-shoot resource competition. These experiments were designed to assess whether supplementary pollination and/or the removal of proximal flowers affects the fruit set of distal flowers (by freeing up resources). The fruit set of distal flowers on control plants was 20%, versus 40%, 86% and 75% for distal flowers on shoots subjected to supplementary pollination, flower removal or both treatments together, respectively (range 0–100% in all cases).

The results of logistic regression show that only proximal flower removal had a significant effect on the probability that a distal flower will set fruit, while neither supplementary pollination nor the interaction between the two factors had a significant effect (Table 1). Flower removal, with or without supplementary pollination, improved the fruit set of distal flowers to levels similar to those of proximal flowers in non-manipulated shoots (*G* = 0.147, *df* = 1, *p* = 0.701).

The results of the analysis of variance show that neither flower removal nor supplementary pollen nor both had a significant effect on fruit weight (mean fruit set weight 0.104 g, 0.137 g, and 0.109 g, respectively).

Discussion

The present results demonstrate that the sequence of flower opening on shoots of *Polygonatum odoratum* (acropetal, i.e., proximal to distal) is paralleled by a decline in fruit set. In our discussion of the possible causes of this pattern, two methodological limitations need to be borne in mind. First we have considered only fruit set as an indicator of female fecundity: fruit set is a useful and important indicator, but other indicators (ovule number, seed number, fruit weight) are also relevant, and may be governed by different factors (possibly including architectural constraints). Second, *P. odoratum* shows an unusual pattern of sex expression, in which some plants bear unisexual male flowers at the distal extreme of the shoot. In view of this, in the present study we used only plants with only hermaphroditic flowers.

The non-uniform pollination hypothesis. Under open pollination, the basal flowers of flowering shoots of *Polygonatum odoratum* have a higher probability of setting fruit than distal flowers. These results are in agreement with previous studies of other hermaphroditic species with flowers grouped in inflorescences (see Medrano et al., 2000^[20] and references therein). Variations in the amount of pollen received did not explain the observed differences in fruit set between proximal and distal flowers, because the differences were maintained when abundant outcross pollen was supplied every day to all open

flowers. Similar results have been obtained with many other species (Holtsford, 1985^[16]; Devlin, 1989^[6]; Karoly, 1992^[17]; Brunet, 1996^[4]; Medrano et al., 2000^[20]). In some species, by contrast, within-inflorescence patterns of fruit and seed production have been shown to be due to variation in the amount of pollen received (Berry and Calvo, 1991^[3]; Goldingay and Whelan, 1993^[11]).

Another possible cause for the observed reduction in fruit set or seed production is variation in pollen "quality" among flowers in different positions. In *P. odoratum*, insects tend to visit proximal flowers first, then distal flowers (data not shown), as has been observed in other bumblebee-pollinated species with sequentially opening flowers (see Brunet and Charlesworth, 1995^[5]). In accordance with this, and given the possible temporal overlap of the male and female phases at different positions on the plant, the "quality" of the pollen received (i.e., the ratio of outcross to geitonogamous pollen) may be influenced by flower position, giving rise to differences in female reproductive success between proximal and distal flowers (see for example Wyatt, 1982^[33]; Berry and Calvo, 1991^[3]; Brunet and Charlesworth, 1995^[5]; Brunet, 1996^[4]). Although we have not specifically studied this possibility, it seems unlikely given that supply of xenogamous pollen to all flowers maintained the pattern of fruit set observed under natural conditions.

Resource competition versus architectural effects hypothesis. Having ruled out the possibility that the observed among-position variations in fruit set are due to differences in the amount or quality of pollen received, two non-exclusive alternative explanations remain: first, that proximal flowers out-compete distal flowers for available resources, and second, that these are intrinsic architectural limitations on fruit set (for example, that the vascular supply to more distal flowers is reduced as consequence of the indeterminate growth of floral axis; Wolfe, 1992^[32]; Diggle, 1995^[7], 1997^[8]).

In the present study, removal of proximal flowers did not improve fruit weight in the remaining distal flowers; however, fruit and seed set were significantly increased, suggesting that the flowers in a flowering shoot compete for resources. Additionally, our present findings indicate that removal of early flowers increases the fruit set of later flowers to levels similar to those of the early flowers of the control flowering shoot, arguing against the architectural effects hypothesis for *P. odoratum*. Similar results have been obtained for other species (Stephenson, 1980^[25]; Herrera, 1991^[15]; Vaughton, 1993^[31]; for a recent review see Medrano et al., 2000^[20]).

In view of these findings, the proximate cause of the reduced fruit set of distal flowers of *P. odoratum* must be considered to be limited resource availability. Possible ultimate causes of this excess flower production include 1) increased attraction of pollinators to the plant or inflorescence as a whole, 2) the possibility of abortion of poor quality fruits, 3) the availability of excess flowers in case of an exceptionally favourable year, 4) the availability of excess flowers in case of loss or damage to others, and 5) increased pollen production (i.e., male function) (Stephenson, 1981^[26]; Sutherland, 1987^[28]; Ehrlén, 1993^[10]; Guitián, 1993^[12]; Stephenson, 1992^[27]).

The first explanation requires a limited pollen supply and, as a result, should be ruled out if supplementary pollination of all flowers on the plant does not affect the pattern of fruit set, as was found in the present study and in previous studies of various other species (Holtsford, 1985^[16]; Karoly, 1992^[17]; Guitián, 1993^[12]). The second explanation likewise does not seem to be applicable to species like *P. odoratum* in which flowering shows a clear spatial sequence, with early flowers showing consistently higher fruit set. The third and fourth explanations have not been specifically tested in the present study, but may be applicable in the case of *P. odoratum*, in which removal of proximal flowers improved the fruit set of more distal flowers. Finally, the fifth explanation may likewise be applicable in *P. odoratum*, since in distal flowers the style is sometimes absent or non-functional (data not shown), and since the mean number of ovules per flower is significantly lower in distal flowers than in proximal flowers (see Results). In dichogamous plants with sequential flowering and consistent directionality of pollinator movement, we can expect sexual specialization of flowers depending on position, which may in turn explain observed patterns of fruit set (see Brunet and Charlesworth, 1995^[5]; Brunet, 1996^[4]).

In conclusion, the results of the present study show that the observed variations in fecundity among flowers at different positions on *P. odoratum* flowering shoots are not attributable to pollen limitations or to architectural effects. Instead, they are at least partially attributable to among-flower competition for limited resources. The ultimate causes of the production of excess flowers are presumably related to the existence of sexual specialization, in turn related to dichogamy and pollinator behaviour. We are currently investigating these questions in greater detail.

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J. Guitián

Departamento de Biología Vegetal
Universidad de Santiago
Laboratorio de Botánica (Farmacia)
Campus Sur s/n
15782 Santiago de Compostela
Spain

E-mail: bvjguiti@usc.es

Section Editor: G. Gottsberger