

Is the dichogamy of *Salvia verbenaca* (*Lamiaceae*) an effective barrier to self-fertilization?

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Abstract: Though dichogamy is generally interpreted as a means of preventing self-fertilization, the efficiency of this mechanism has been questioned. Little attention has been paid to functional aspects of male/female timing, such as variation over time in pollen germinability and in stigma receptivity. In the present study these aspects of male/female timing were investigated in the protandrous species *Salvia verbenaca*. To evaluate the effects of selfing, fruit set, total seed weight produced and seed/ovule ratio were also determined, (a) following artificial crossing, (b) following selfing (i.e. bagging) and (c) under natural conditions. Both pollen germinability and stigma receptivity peaked on the last day (day 3) of the flower's life, which presumably favours selfing. Neither fruit set nor seed/ovule ratio were significantly affected by pollination regime, but the total seed weight produced by artificially crossed plants was significantly higher than that produced by bagged or untreated plants. Neither fruit set, seed/ovule ratio nor total seed weight differed significantly between the two years of study. Thus, if a *S. verbenaca* flower receives outcrossing pollen it will produce a greater weight of seeds (with consequent advantages in terms of female reproductive success). However, the peaking of both pollen germinability and stigma receptivity on day 3 of the flower's life means that selfing is likely to occur if outcrossing pollen has not been received over the early part of the flower's life.

In flowering plants, the male and female organs are frequently separated in space (hercogamy) or time (dichogamy). Such characteristics have generally been interpreted as mechanisms for the prevention of self-fertilization (see for example FAEGRI & VAN DER PIJL 1979). However, the effectiveness of these mechanisms has been disputed. Over a century ago, MÜLLER (1883) questioned whether the dichogamy of *Scrophularia nodosa* L. was an effective barrier to self-fertilization, and a number of more recent studies have likewise cast doubt on the efficiency of dichogamy (NYMAN 1992, ORTEGA-OLIVENCIA & DEVESA 1993). For example, it is difficult to explain dichogamy in species which are physiologically self-incompatible, unless the physiological self-incompatibility is only partial (see LLOYD & WEBB 1986) or arose after protandry or protogyny (FAEGRI & VAN DER PIJL 1979). Indeed, some authors have suggested that dichogamy may be important for

other reasons (e.g. to avoid pollen-stigma interference) (LLOYD & WEBB 1986, BERTIN 1993, BERTIN & NEWMAN 1993). However, the development of general hypotheses to explain the observed diversity of sexual systems should be based not only on morphology but also on functional aspects (BAWA & BEACH 1981).

Flowers are traditionally defined as protandrous when pollen release precedes the onset of stigma receptivity, and as protogynous in the reverse case (PERCIVAL 1965). Little attention has been paid to other aspects of pollen dispersal and stigma receptivity, such as the length of time between pollen release and the onset of stigma receptivity, the degree of opening synchrony among flowers on the same plant, and variability over time in pollen germinability and in stigma receptivity (though see WEBB 1981, and LLOYD & WEBB 1986, for discussion of different types of dichogamy). These aspects of dichogamy, however, may have played a key role in the evolution of protandry or protogyny as barriers to self-fertilization.

Protandry is common in the *Labiatae* (OWENS & UBERA-JIMENEZ 1992), but its effectiveness as a barrier to self-fertilization differs between species. For example, protandry effectively prevents self-fertilization in *Lavandula stoechas* L. (MUÑOZ & DEVESA 1987), but not in species of the genus *Nepeta*, in which pollen release and stigma receptivity are effectively separated in time but in which self-fertilization is not precluded because of within-inflorescence geitonogamy (UBERA & VALDES 1983).

In a study of reproductive systems in the genus *Salvia*, HAQUE & GHOSHAL (1981) distinguished two trends: species with large, red/scarlet, nectar-producing, mainly heterostylous flowers reproducing by xenogamy, and species with small, blueish, homostylous flowers reproducing by autogamy. These authors assigned *Salvia verbenaca* L. to the second group, in view of its autogamous reproduction; however, this is difficult to reconcile with the fact that the flowers are protandrous.

The objective of the present study was to investigate the effectiveness of the dichogamy of *Salvia verbenaca* for preventing self-fertilization. To this end, I quantified (a) temporal variability in pollen germinability and (b) the length of the period of stigma receptivity. I also examined the effect of self- and cross-pollination on fruit set and seed production (number and weight).

Materials and methods

The plant and the study area. *Salvia verbenaca* (*Lamiaceae*) is a 30–60 cm high biennial herb with blueish purple flowers of about 1 cm length arranged in verticillasters (each of which generally contains six flowers). The calyx (green, 4–8 mm long) encloses a 6–10 mm long corolla. The flowers have traditionally been defined as protandrous, as pollen is released before the stigma becomes receptive. Within each plant, flowering is asynchronous, flowering order being acropetal. Nutlet fruits contained 1–4 seeds. The verticillasters are close together on the stem at flowering, but move further apart by fruit set. Flowering commences in mid-April and finishes towards the end of May. The fruits mature (allowing seed release) about two weeks after senescence of the flower. Pollination is largely by the honey bee *Apis mellifera* L. and *Bombyliidae* such as *Bombylius major* L. (pers. obs.). Each flower produces 0.6–1.5 μ l of nectar per day (PETANIDOU & VOKOU 1993).

The study was carried out in spring 1993 and spring 1994 in Vilardesilva, in the far western part of the El Bierzo region, close to the Galicia-León border in NW Spain. The study population is located in an area of seminatural pasture over limestone.

Flower duration. *Salvia verbenaca* flower duration has been investigated previously in another study area (PETANIDOU & VOKOU 1993); I made independent observations because geographical variation in flower duration has been reported for a number of species (see PRIMACK 1985a). I tagged 15 flower buds (each on a different plant) and monitored them daily throughout the flowering period, recording the day of opening and the day of abscission of each flower. I also noted stamen, style and stigma position daily, and recorded the day of anther dehiscence.

Pollen germinability. In spring 1993 I collected all pollen from one-, two- and three-day-old flowers which had been bagged before opening to prevent receipt of pollen from other flowers ($n = 10$ flowers by age class). All pollen from each flower was collected and immediately placed in petri dishes on a sterile solid medium (BAR-SHALOM & MATTSO 1977) containing 30% sucrose (this being the concentration which gave the highest germination rate in preliminary tests with 5, 10, 20, 30 and 40% sucrose) and incubated for 4 h. Germination counts were done under a stereomicroscope, all grains with a pollen tube of length greater than grain diameter being counted as 'germinated'.

Stigma receptivity. To investigate stigma receptivity, in spring 1993 I hand-pollinated one-, two- and three-day-old flowers with pollen from other plants ($n = 10$ flowers by age class). Bagged flowers on the recipient plant were carefully emasculated at anthesis, then manually pollinated. Fruit formation was recorded about two weeks later.

Fruit set and seed production after autogamy and after xenogamy. I hand-pollinated all flowers on four randomly selected plants with pollen from other plants. At the same time another four randomly selected plants were bagged with mosquito netting to impede pollinator access; these plants were left bagged until senescence of the last flower. Finally, I selected 10 control plants (near to the treated plants) which were not interfered with in any way. In spring 1994 I marked another 10 plants in the same population as that year's control. Fruits were collected about two weeks after senescence of each flower, allowing calculation for each experimental group (hand-pollinated, bagged and control plants) of mean fruit-set level per plant, mean seed/ovule ratio of the fruits obtained after each treatment and mean weight of the seeds obtained after each treatment. Note that seed/ovule ratio is easily determined in this species because non-fertilized ovules are clearly identifiable.

Statistical analysis. Variability in pollen germinability over time was investigated by Spearman's rank correlation analysis. The effects of hand-pollination and bagging on seed production were investigated by analysis of variance, with Tukey's test for subsequent multiple comparisons. Percentage seed/ovule ratio data were first subjected to an arcsine transformation. The effects of hand pollination and bagging on fruit set were investigated with the aid of the Mann-Whitney U test.

Results

Mean flower duration was 2.9 ± 0.3 days ($n = 15$). As expected, flowers were protandrous, with two slightly exerted stamens which in all cases dehisced on the first day of anthesis. The switch from the male to the female phase was signalled by elongation of the style from the second day post-opening onwards.

Pollen germinability increased steadily with days since anthesis ($r_S = 0.943$, $p < 0.001$). Both pollen germinability and stigma receptivity peaked on day 3 post-anthesis (Fig. 1).

Fruit-set was high in all experimental groups (hand-pollinated, bagged and control; Table 1), the bagged plants had a slightly lower fruit set than the control plants ($U = 1.0$, d.f. = 1, $p < 0.05$). Seed/ovule ratio was high and did not vary

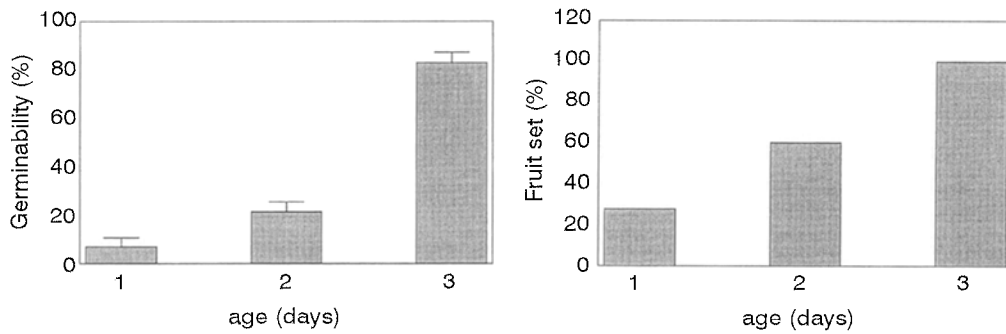


Fig. 1. Left: Germinability (mean \pm s. d.) of pollen grains of *Salvia verbenaca* collected on day 1, 2 and 3. Germinability is expressed as percentage of the total number of pollen grains collected from the flower. Right: Stigma receptivity of *Salvia verbenaca* flowers of different age, estimated as percentage of flowers setting fruit following hand-crossing on day 1, 2 or 3

Table 1. Fruit set per plant (mean \pm s.d., sample sizes in brackets), seed/ovule ratio and seed weight for bagged, hand-crossed and control *Salvia verbenaca* plants. Within a column, values with the same superscript do not differ significantly (Tukey's test)

Treatment	Fruit set (%)	Seeds/ovules (%)	Seed weight (μg)
Bagged	94.2 \pm 4.5 (4)	83.5 \pm 21.4 (194) ^a	1718 \pm 634 (120) ^b
Hand-crossed	96.0 \pm 2.0 (4)	78.7 \pm 26.9 (227) ^a	2071 \pm 481 (120) ^c
Control 1993	99.4 \pm 0.8 (10)	82.6 \pm 19.5 (237) ^a	1787 \pm 690 (2140) ^b

significantly among groups ($F = 2.3$, d.f. = 2, n.s.). However, mean weight of seeds produced by hand-pollinated plants was significantly higher than that produced by bagged and control plants ($F = 10.8$, d.f. = 2, $p < 0.001$).

I did not detect any significant between-year variation in fruit-set (99.4 \pm 0.8% in 1993, 99.3 \pm 1.0% in 1994; $F = 0.001$, d.f. = 0.1, n.s.), seed/ovule ratio (82.6 \pm 19.5 in 1993, 80.7 \pm 23.1 in 1994; $F = 0.2$, d.f. = 1, n.s.) or weight of seed produced per plant (1787 \pm 690 μg in 1993; 1813 \pm 692 μg in 1994; $F = 1.4$, d.f. = 1, n.s.).

Discussion

A flower's longevity is one of the most important determinants of the number of pollinator visits received by that flowers (PRIMACK 1985b). In a given species, flower longevity may vary within the same plant (MUÑOZ & DEVESA 1987), between populations (PRIMACK 1985a) or throughout the flowering season (NAVARRO & al. 1993). In the case of *Salvia verbenaca*, the longevity of flowers in the study area was similar to that reported for other populations in an East Mediterranean area by PETANIDOU & VOKOU (1993).

I found that stigma receptivity increased with flower age, as expected for a protandrous species. The relationship between flower age and pollen germinability was, however, the reverse of that expected, and in fact the moment of maximum

pollen germinability coincided with the moment of maximum stigma receptivity (see Fig. 1). Thus, despite the fact that *Salvia verbenaca* is protandrous, the timing of maximum pollen germinability and stigma receptivity would appear to allow self-fertilization. This may be an adaptation to allow self-fertilization when cross-pollination fails (i.e. deferred self-pollination sensu LLOYD & SCHOEN 1992).

Salvia verbenaca had very high fruit set levels and seed/ovule ratios (Table 1). Fertilization did not require insects and, as reported by HAQUE & GHOSHAL (1981), this species is self-compatible. However, the mean weight of seeds produced by artificially cross-pollinated flowers was about 20% higher than that produced by bagged (i.e. self-pollinated) flowers; cross-pollination is thus clearly advantageous (assuming, of course, that heavy seeds are advantageous; see NAVARRO 1996). These findings thus suggest the *Salvia verbenaca* is a facultative xenogam.

The results of this study are similar to those obtained by LYON (1992) for *Sanguinaria canadensis* L., a spring-flowering species in which xenogamy is facultative: flowers are cross-pollinated when pollinator activity is sufficient but self-fertilized if pollinator activity is reduced due to cold or wet weather. Similar breeding-system plasticity has also been reported for a number of other species (see JUNCOSA & WEBSTER 1989). WYATT (1984), in a study of nine populations of *Arenaria uniflora* (WALTER) MUHLENB., found a full spectrum from predominant outcrossing to predominant selfing, with varying potential for spontaneous self-pollination. He also noted that, in some populations, self-pollination was prevented by strong protandry. Similar patterns have been reported for *Lupinus texensis* HOOK. (SCHAAL & LEVERICH 1980) and *Lavandula stoechas* (MUÑOZ & DEVESA 1987), in which pollen viability rapidly decreases after the male phase. NYMAN (1992), in a study of the genus *Campanula*, concluded that pollen germination patterns may favour selfing, crossing or any of various combined strategies. In *Campanula dichotoma* L., for example, autogamy is not precluded as a secondary mating system to ensure pollination in the absence of insect visits, since self pollen remains viable for some time. Similarly, LYON (1992) has suggested that facultative xenogamy is often a good strategy for plants which flower in early spring, when the weather is difficult to predict and pollinators may be inactive; such a strategy permits cross-pollination when pollinators are active, but allows for self-pollination when pollen receipt is limiting. *Salvia verbenaca* flowers early in the year, when pollinator activity in the study area is low. My observations suggest that pollinator outcrossing activity was indeed limiting: seed weight produced by non-manipulated plants was similar to that produced by bagged plants, and seed weight in both cases was lower than that produced by artificially crossed plants. Intraspecific variability in seed weight produced can be expected to have important evolutionary implications, since seed weight may affect germination time, germination rate and seedling survival, and is likely to influence the outcome of intra- or interspecific competition (see NAVARRO 1996).

In conclusion, the results of the present study, together with those of NYMAN (1992) for *Campanula dichotoma*, support the hypothesis that the occurrence or non-occurrence of selfing in protandrous species is more dependent on pollen germinability patterns than on the temporal separation of the stamen dehiscence and stylar elongation phases. Investigation of pollen germinability and stigma receptivity is thus likely to prove indispensable for elucidating mechanisms of self-

and cross-compatibility in dichogamous species. Moreover, greater attention should be paid to other possible functions of protandry: for example, it may impede blockage of stigmas with self-pollen, and/or blockage of styles and micropyles with self-pollen tubes (see BERTIN 1993, and references therein).

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