Report on the outcomes of a Virtual Mobility[1]

Action number: CA18201 ConservePlants

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Virtual Mobility Details

Title: Reproductive strategies of the endemic *Salvia saccardiana* and strength of its reproductive barriers with *S. pratensis.*

Start and end date: 18/03/2024 to 05/04/2024

Description of the work carried out during the VM

(max. 500 words)

Despite natural hybridization has long been recognized as a means for gene flow between taxa bringing important evolutionary consequences, one of the possible outcomes from introgressive hybridization includes the loss of one of the parental forms through genetic assimilation by the other.
Salvia pratensis and its narrow endemic congener *S. saccardiana* are two ecologically distinct species that can, occasionally, grow in close contact. The two species differ markedly physiognomically, karyologically, and ecologically. More specifically, the two show different habitat preferences: *S. saccardiana* is more commonly found in a thermophytic fringe vegetation on dolomitic bedrock, while *S. pratensis* prefers dry to mesophytic grasslands on limestone. Despite they differ markedly in flower size, the study by Balant et al. conducted in 2019 reported a relatively low number of intermediate individuals, whose intermediate flower sizes could either be ascribable to bedrock influence or hybridization. During this Virtual Mobility Grant period, I analysed data collected during my previous STSM Grant (May 2023) and before. During this period, I tried to answer the following research questions: i) whether intermediate flower sizes may be a consequence of bedrock type, and ii) which reproductive barriers (if any) intervene in isolating the two species. During the Virtual Mobility period, I then drafted a manuscript resumed in the graphical abstract below (Figure 1).

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

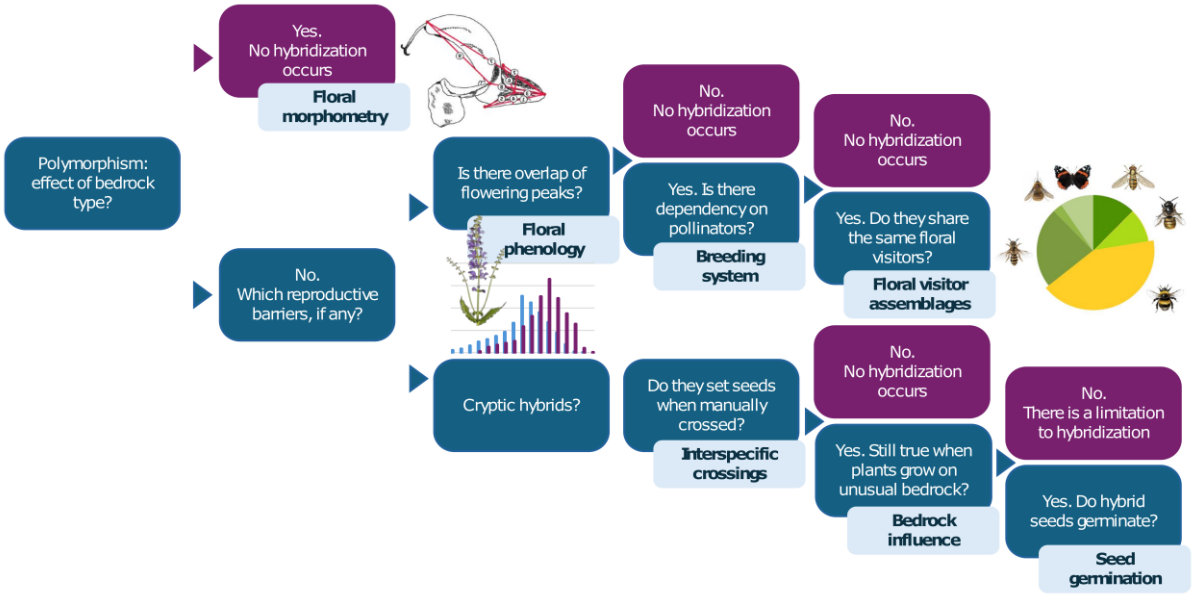

Figure 1. Graphical abstract resuming the narrative of the manuscript drafted during the Virtual Mobility Grant period.

Our results on flower morphometry performed on individuals transplanted either on their habitual or opposite bedrock type confirm distinct separation of flower sizes of the two species, with no pronounced effect induced by bedrock (Figure 2).

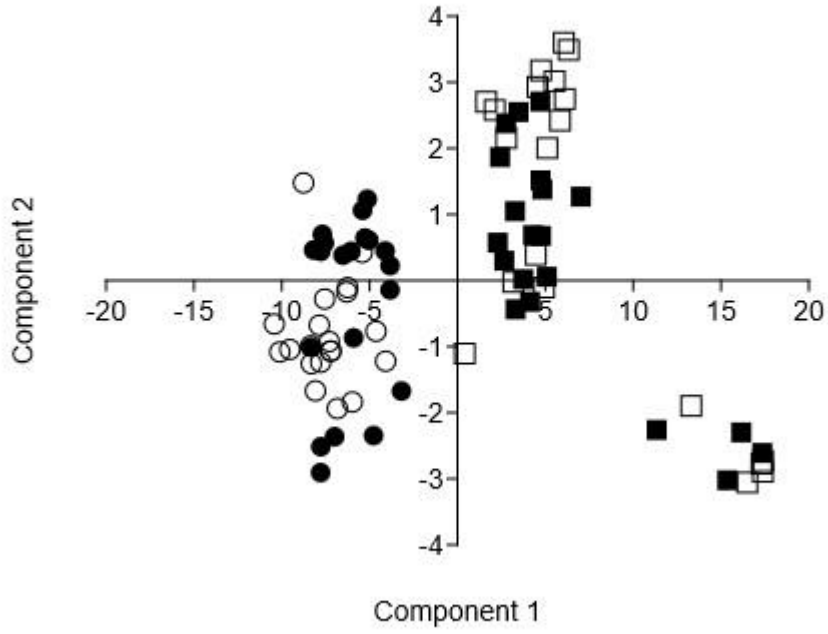

Figure 2. Principal components analysis (PCA) on floral morphometrical parameters. *Salvia pratensis* is indicated with circles, while *S. saccardiana* is indicated with squares. Individuals grown on their habitual bedrock (PL and SD, respectively) are indicated by solid symbols, while individuals grown on the opposite bedrock (PD and SL, respectively) are indicated by empty symbols.

As expected, floral phenology did not represent a barrier: both species, in fact, showed an extended flowering period of four weeks, whose peaks were recorded just one week apart.
Similarly to flower phenology, neither flower biology nor mating system were expected to represent strong reproductive barriers, as a high degree of dependency on animal pollinators was predicted for both species. Both species are self-compatible and exhibit weak protandry. Unmanipulated flowers, however, set an extremely low number of seeds, suggesting that self-pollination (within the same flower) is avoided by the lever mechanism, and that both species are strictly dependent on animal pollination.
Our observations on floral visitors revealed that the two species share the same pollinator assemblage. However, finer observations on flower-insect interactions revealed the need to further classify floral visitors in smaller functional groups according to the behavior exhibited on flowers. Each insect taxon was therefore assigned to one out of three functional groups identified based on the most frequent type of interaction with the flower. These categories were: i) pollinator, ii) pollen or nectar thieve, and iii) flower burglar.
When the two species were manually crossed, we observed pronounced asymmetrical hybridization (Figure 3a). Regardless of the soil type where mother and father plants had been grown on, in fact, *Salvia pratensis* was always able to set a good number of seeds. On the contrary, *Salvia saccardiana* in no case was able to set a relevant number of seeds (Figure 3b).

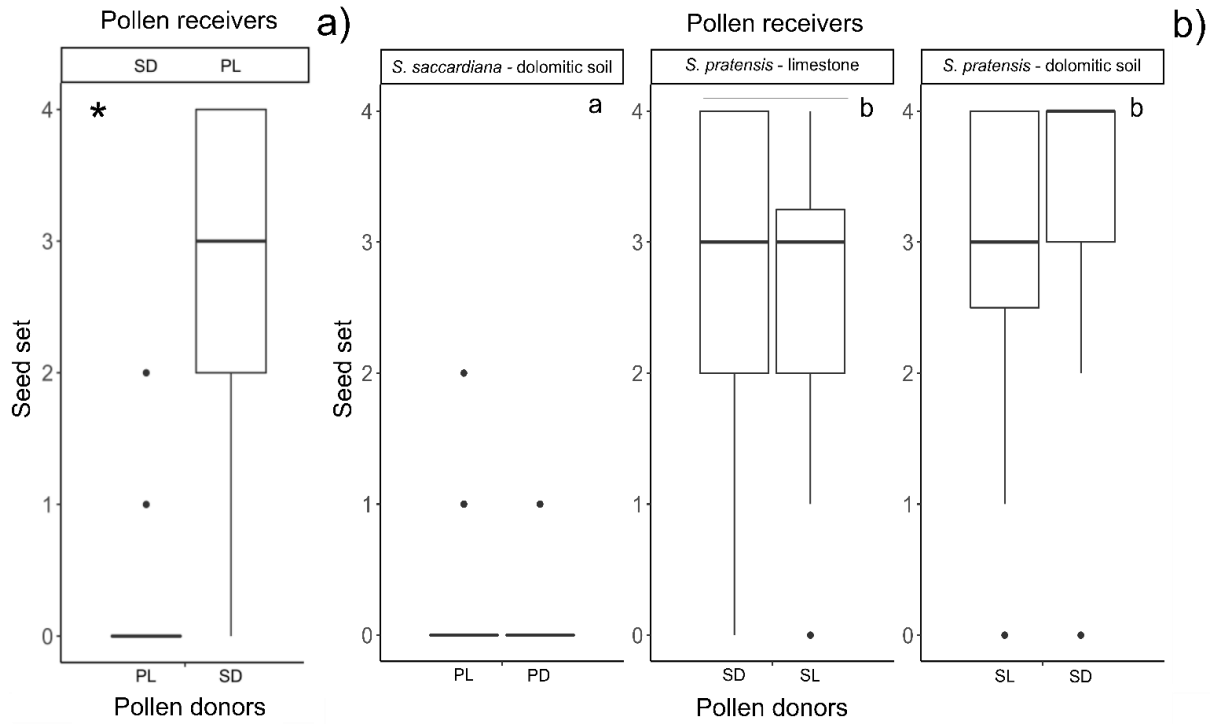

Figure 3. a) no. of seeds set by *Salvia saccardiana* (SD) when pollinated with pollen of *Salvia pratensis* (PL) and viceversa. b) no. of seeds set by *Salvia saccardiana* and *S. pratensis* grown on different bedrock types when pollinated with heterospecific pollen coming from plants grown either on their habitual or opposite bedrock type. SD = *S. saccardiana* on dolomitic bedrock, PL = *S. pratensis* on limestone, SL = *S. saccardiana* on limestone, PD = *S. pratensis* on dolomitic bedrock.

Description of the VM main achievements and planned follow-up activities

(max. 500 words)

The main achievement of this Virtual Mobility Grant period was drafting a manuscript that, when published, will provide the scientific community with important information about mating system, pollinators, and ecology of the narrow endemic species *Salvia saccardiana*. Though part of such results were, somehow predictable, our study is the first reporting results on the reproductive biology of the narrow endemic *Salvia saccardiana*, that had never been investigated before.
Moreover, this work highlights that even though hybridization with the congener species *Salvia pratensis* does occur, this is pronouncedly asymmetrical. As one of the possible outcomes from introgressive hybridization includes the loss of one of the parental forms through genetic assimilation by the other, our results show that strong post-pollination/pre-zygotic barriers preserve *Salvia saccardiana* from introgressive hybridization from the more common and widespread species *Salvia pratensis*.