Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA18201

Grantee name: Manica Balant

Details of the STSM

Title: **Does hybridization with evolutionary closely related but ecologically divergent and widespread species threatens endemic *Salvia saccardiana*?**

Start and end date: 02/05/2022 to 12/05/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

The experimental part of the research was done both in the garden of the Natural History Museum Rijeka (Croatia), and partially in the field (in Brod na Kolpi, Croatia), as previsioned in the project proposal.

We collected 1 hermaphroditic flower from each of the 20 individuals per species present in the garden. The flowers were photographed, and the analysis (using geometric morphometrics approach) of the material will be done in the next months.

We analysed the reproductive biology (flower lifespan, mating system, and floral reward) of both *Salvia saccardiana* and *S. pratensis*. The flower lifespan was done on 20 randomly selected buds per species. In average, the flowers lasted between 3 and 4 days in both species. Pollen viability and stigma receptivity were tested for both species in big buds (19 buds), 1,2,3 and 4 days old flowers (see Table 1). Slides with stained pollen were fixated and are being reviewed at the moment. Stigmas for stigma receptivity were collected 6h after the pollination and fixated in 90% ethanol. They will be reviewed in the next month.

We also measured the nectar volume and concentration in both species for a few flowers, but the values obtained were very unexpected. For example, in *S. saccardiana* grown in the garden we found sugar concentrations above 50%. Additionally, the nectar volume in *S. pratensis* was so low, that was impossible to measure individual flowers. This is why we decided to skip the nectar measurements this year, and focus on the nectar analysis next year.

[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.

Table 1: Number of flowers analysed for sexual functioning

Days old	*S. pratensis*	*S. saccardiana*
Bud	19	19
1	17	26
2	18	22
3	19	20
4	13	9

The breeding system was evaluated for both species. We have performed 138 manipulations in *S. saccardiana* and 151 manipulations in *S. pratensis*. We also performed between species crosses to see if reproductive barriers (pre- or post-zygotic) between the taxa exist. For details see Table 2. The manipulated flowers were marked, and we are waiting for the seeds to develop. The mature seeds will be collected and weighed in the next months. We will germinate them during the winter.

Table 2: Number of flowers analysed for breeding system and between species crosses.

Manipulation	*S. pratensis*	*S. saccardiana*
As	29	28
Ai	32	23
G	32	25
Xe	28	27
Xe_sp	30	35

The fieldwork at the locality Brod na Kolpi was done on two locations in close proximity – in one locality we observed plant-pollinator interactions on *S. saccardiana* (45°28'29.9"N 14°47'55.4"E) and on the other on *S. pratensis* (45°28'01"N 14°48'38"E). Observations were done on three different days. The first two days we were observing and recording the visitors and pollinators on both species between 9:00 and 15:00. On the last day, we collected the plants visitors and pollinators every second hour (starting at 8:00 and finishing at 17:00) and kept them for further identification.

There were no major deviations from the initial plan. The only major change was the change of the start date of the STSM. In the initial work plan, we proposed the STSM to be carried out between the 22/04/2022 and 01/05/2022. However, because of the late flowering of the plants, the beginning of the STSM was postponed for a few days and it was carried out between 02/05/2022 and 12/05/2022. The change of dates was previously approved by Amy Simons in the new Grant Letter.

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

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

The STSM achieved the main goals planed in the initial work plan. We successfully preformed the first part of the experimental work that will enable us to study different aspects of the reproductive biology of *Salvia pratensis* and *Salvia saccardiana*, test the reproductive barriers between the taxa and asses the threats that hybridisation between the two species poses to *S. saccardiana*.

As initially planned, only part of the experimental work was carried out during the STSM. The collaborators from the Natural History Museum Rijeka (Croatia), are continuing with the second part of the experiment (collecting the seeds after the maturation, weighing and germinating them, and revising the slides for pollen viability and stigma receptivity), while the STSM candidate will shortly start with the analysis of the photographic material for the flower morphology analysis and later with the analysis of the results obtained in the study. The study will continue next year, to also include the nectar analysis we were unable to perform this year. After we will obtain all the results, a publication of an article about the reproductive biology of *S. pratensis* and *S. saccardiana* is planned.