Finding Bermuda buttercup (*Oxalis pes-caprae*):

understanding the evolutionary and ecological processes and patterns of biological invasions in the Mediterranean region

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INTRODUCTION

Oxalis pes-caprae, a tristylous plant native to South Africa with a complex reproductive strategy, is an invasive species in Mediterranean climates worldwide. While in its native area the populations are represented by all three flower morphs, which enables the plant to reproduce sexually, populations in the invaded ranges are less diverse, composed (predominantly) of short-styled morphs and reproduce asexually. However, latest research in the W Mediterranean has shown the presence of other floral morphs in some invasive populations, as well as occasional sexual reproduction.

OBJECTIVE

The aim of this study is to better understand the changes in the reproductive system of *O. pes-caprae* in the entire Mediterranean basin.

Figure 1: Flowers of *O. pes-caprae* and its different flower morphs. Arrows point to the stigma, giving the name to the respective morph.

MATERIALS & METHODS

Plant samples were obtained from 140 populations from 12 countries within the Mediterranean area. We focused on two aspects of reproductive biology: the flower morphology and the receptivity of the stigma. For the morphometric study, we used 10 flowers per population and measured the hight of both stamen circles, the stigma, and the size of corolla (Figure 2). To prepare samples for the analysis of stigma receptivity, we followed the protocol by Costa et al. (2014). 15 samples per population were observed under the fluorescent microscope with UV-1A filter. For each flower, we counted the number of pollen grains on the stigma and the number of pollen tubes at various depths within the style.

Figure 2: Left: Samples of flowers prepared for the morphometric study. Right: measurements of different flower parts. The colours correspond to colours in Figure 4.

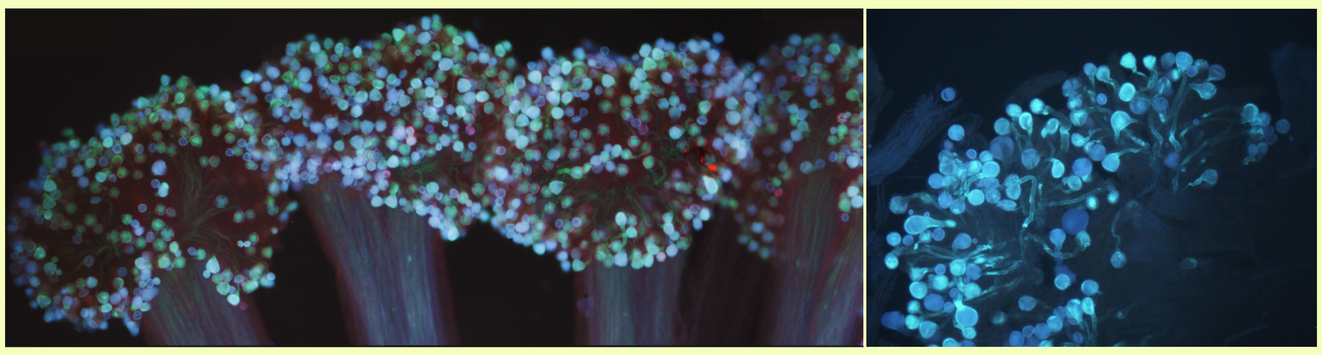
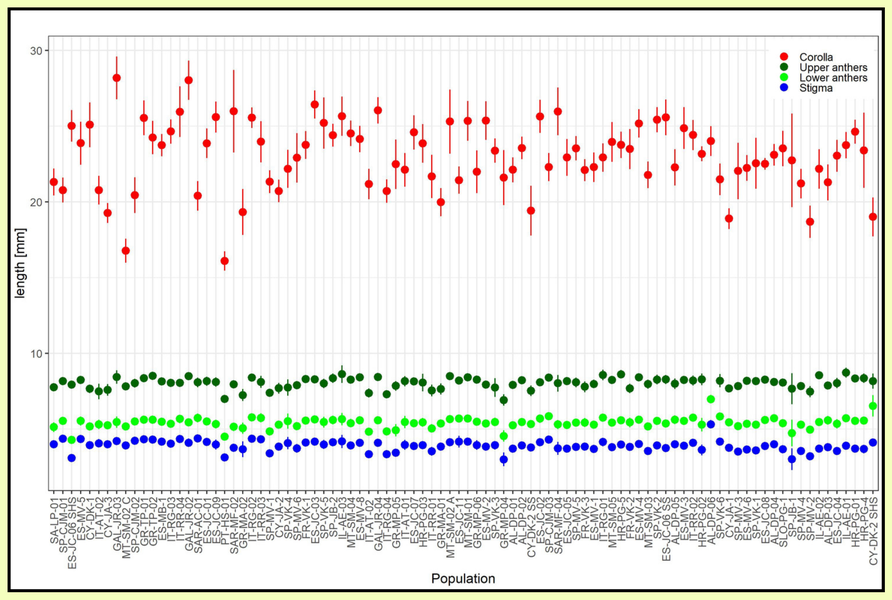


Figure 3: Anniline-stained stigmas observed under fluorescent light. Abundant deposition of pollen grains is visible, as well as many pollen tubes protruding into the style.

RESULTS & DISCUSSION

The preliminary results of our study show that most stigmas are abundantly covered with pollen grains and pollen tubes were frequently observed in the upper part of the style (Table 1). However, pollen tubes protruding into the lower part of the style and reaching the ovary occurred only in a small number of samples. This was mainly observed in individuals where the distance between the stigma and the lower anthers was small. Here we show only the 55 samples with the smallest distance between stigma and lower anthers (Figure 4). To assess pollen germination on stigmas we focused on populations were the morphological changes were already documented. However, further analysis of other populations is still required. The breakdown of incompatibility probably occurs in a limited number of individuals with a smaller distance between stigma and lower anther (Figure 4). As this was only observed at the individual level, we cannot draw conclusions for the population level. We did observe that the pollen tube reached the ovary, but this only happened in a small number of cases.



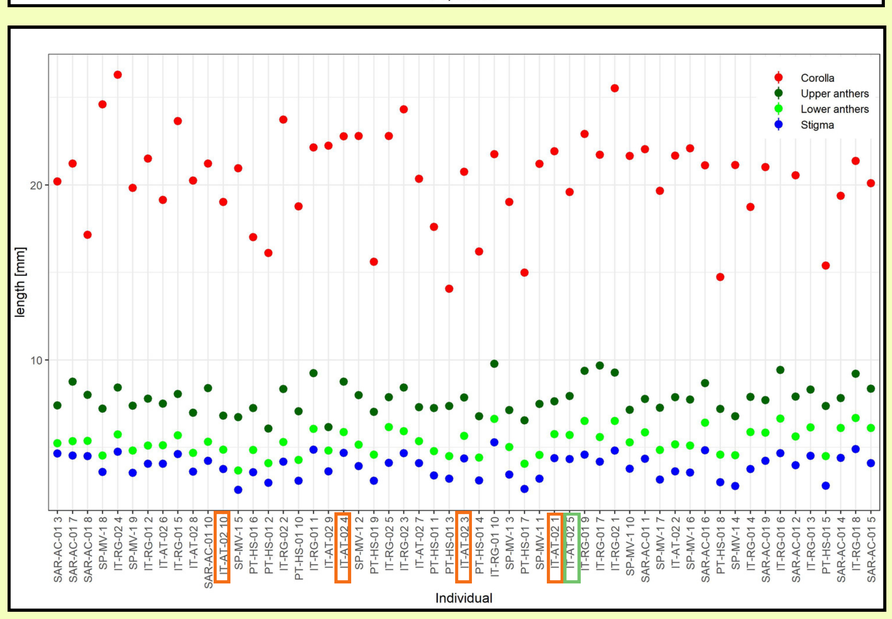


Figure 4: Above: Average length of lower and upper anthers, stigma and corolla per population. Populations are ordered according to the increasing size between the position of the stigma and the lower anthers. Below: length of lower and upper anthers, stigma and corolla per individual, ordered as in fig. above. Only the first 55 individuals are shown. For individuals marked with orange and green, pollen tubes reaching the middle and lower part of style, respectively, were reported.

Population	Samples per population	Floral morph	No. of pollen grains on stigma	% of germinated pollen grains	Pollen tubes in upper part of style	Pollen tubes in lower part of style (reaching ovary)
SAR- AC-01	13	S	285,9 (165,1)	29,9	59,9 (58,4)	0,0 (0,0)
SAR-AC-03	15	S	361,1 (200,2)	52,1	188,4 (123,1)	0,0 (0,0)
IT-AT-02	15	S	109,4 (122,2)	31,0	33,9 (45,8)	0,0 (0,0)
SP-MV-1	15	S	189,9 (169,1)	44,9	85,2 (93,0)	0,0 (0,0)
IT-RG-02	15	S	159,3 (130,0)	33,6	53,5 (63,5)	0,0 (0,0)
SA-LP-01	15	S	209,7 (222,8)	55,3	100,8 (133,4)	0,0 (0,0)
IT-RG-04	15	S	344,9 (180,3)	50,0	172,3 (110,4)	0,0 (0,0)
MT-SM-01-XAGHRA	15	S	303,1 (195,0)	61,4	178,1 (125,7)	**0,1** (**0,3**)
MT-SM-02 A1-10	7	S	288,6 (234,8)	30,4	87,9 (62,8)	0,0 (0,0)
AL-DP-03	15	S	407,8 (300,1)	60,9	223,5 (185,2)	0,0 (0,0)
ES-JC-06	15	S	73,5 (70,1)	41,0	29,2 (43,2)	0,0 (0,0)
GR-TP-01	15	S	228,1 (219,1)	40,1	91,5 (56,0)	0,0 (0,0)
MT-JB-03	15	S	197,3 (228,1)	60,0	118,1 (68,2)	0,0 (0,0)
CY-DK-02	15	S	55,5 (78,0)	35,9	19,9 (34,1)	0,0 (0,0)
ES-JC-06	15	SHS	275,5 (228,4)	33,8	81,6 (93,9)	**3,2** (**6,3**)
CY-DK-02	6	SHS	243,2 (148,7)	40,1	86,0 (78,8)	0,0 (0,0)
IT-AT-02	15	M	155,0 (253,6)	38,6	46,8 (68,0)	**0,2** (**0,8**)
PT-HS-01	15	LS	393,0 (442,7)	26,3	103,3 (165,9)	0,0 (0,0)

Table 1: Deposition and germination of pollen grains on stigmas. Standard deviation is given in brackets. Floral morph codes: S - short styled, SHS - semi-homostylous, M - medium styled, L - long styled. Country codes: AL - Albania, CY - Cyprus, ES / SP - Spain, GR - Greece, IT - Italy, MT - Malta, SAR - Italy (Sardegna), PT - Portugal.

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