Finding Bermuda buttercup

Shifts in reproductive traits after introduction in the Mediterranean basin

Sílvia Castro[1], Peter Glasnović[2] *et al.*

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[2]Natural Sciences and Information Technologies • Faculty of Mathematics • University of Primorska, Koper, Slovenia

CONSERVE Plants

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UNIVERZA NA PRIMORSKEM UNIVERSITÀ DEL LITORALE

FLOWERLAB

CENTRE FOR FUNCTIONAL ECOLOGY SCIENCE FOR PEOPLE & THE PLANET

1290 UNIVERSIDADE DE COIMBRA DEPARTAMENTO DE CIÊNCIAS DA VIDA

STUDY SYSTEM: ***Oxalis pes-caprae* L.**

Geophyte

Asexual reproduction

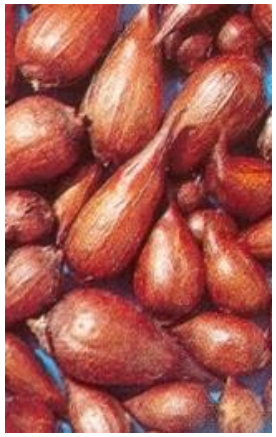
- High production of bulbs
- Root contraction capacities

Sexual reproduction

- Tristylous species

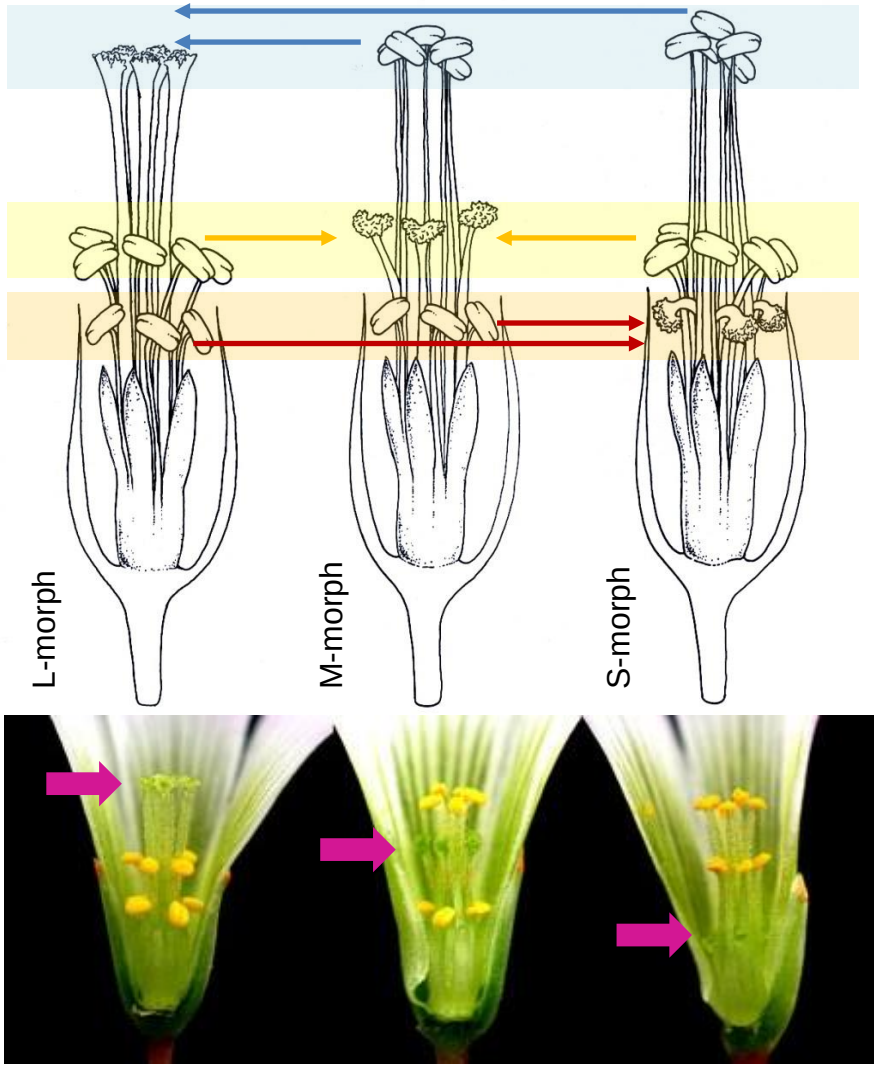
Polyploid complex

- 2*x*, 4*x* and 5*x* cytotypes



STUDY SYSTEM: *Oxalis pes-caprae* L.

Sexual system



Tristylous species

- Long-styled morph
- Mid-styled morph
- Short-styled morph

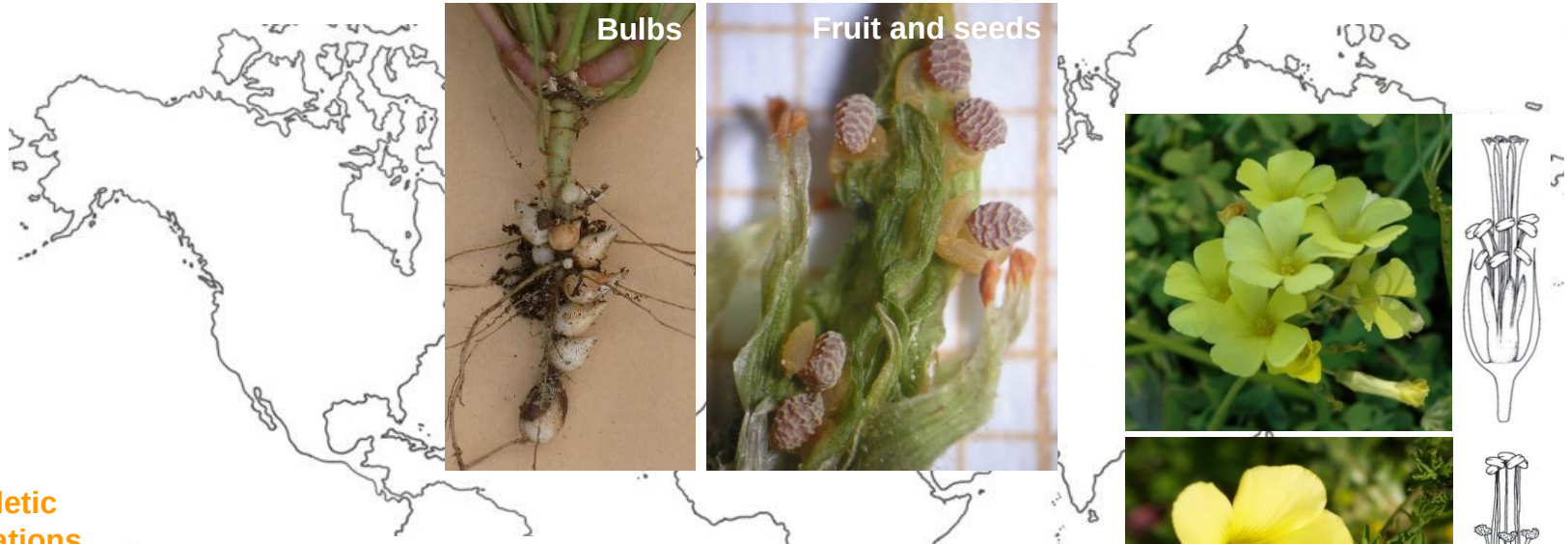
Trimorphic incompatibility

(i.e., self and intra-morph incompatible)

Ornduff R. 1987. Annals of Missouri Botanical Garden 74:79-84.

Oxalis pes-caprae L.: Distribution patterns and diversity of forms

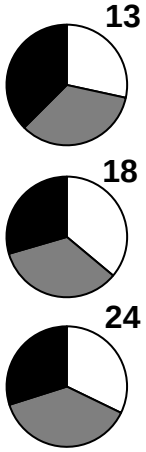
Native area

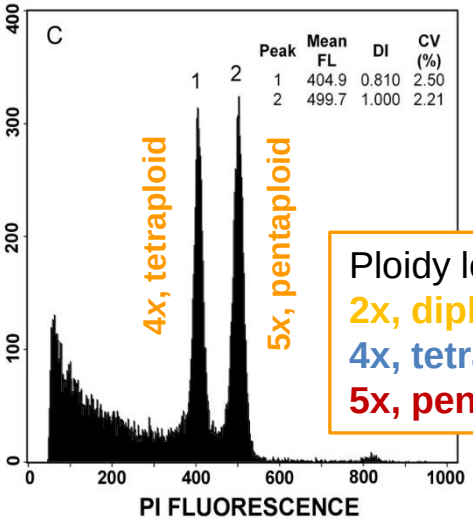


L-morph
M-morph
S-morph

South Africa

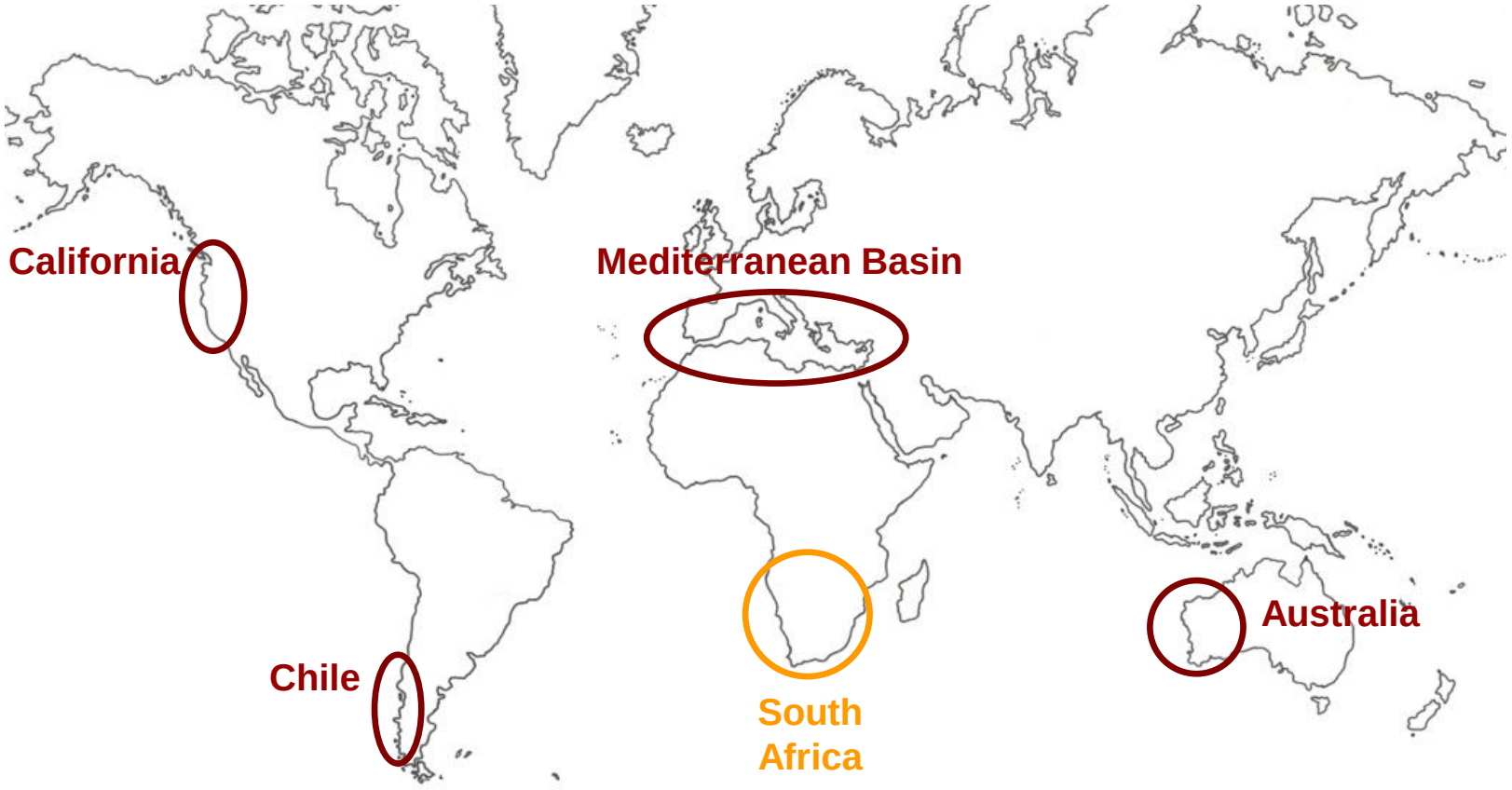
Isopletic populations





Ploidy levels:
2x, diploid
4x, tetraploid
5x, pentaploid

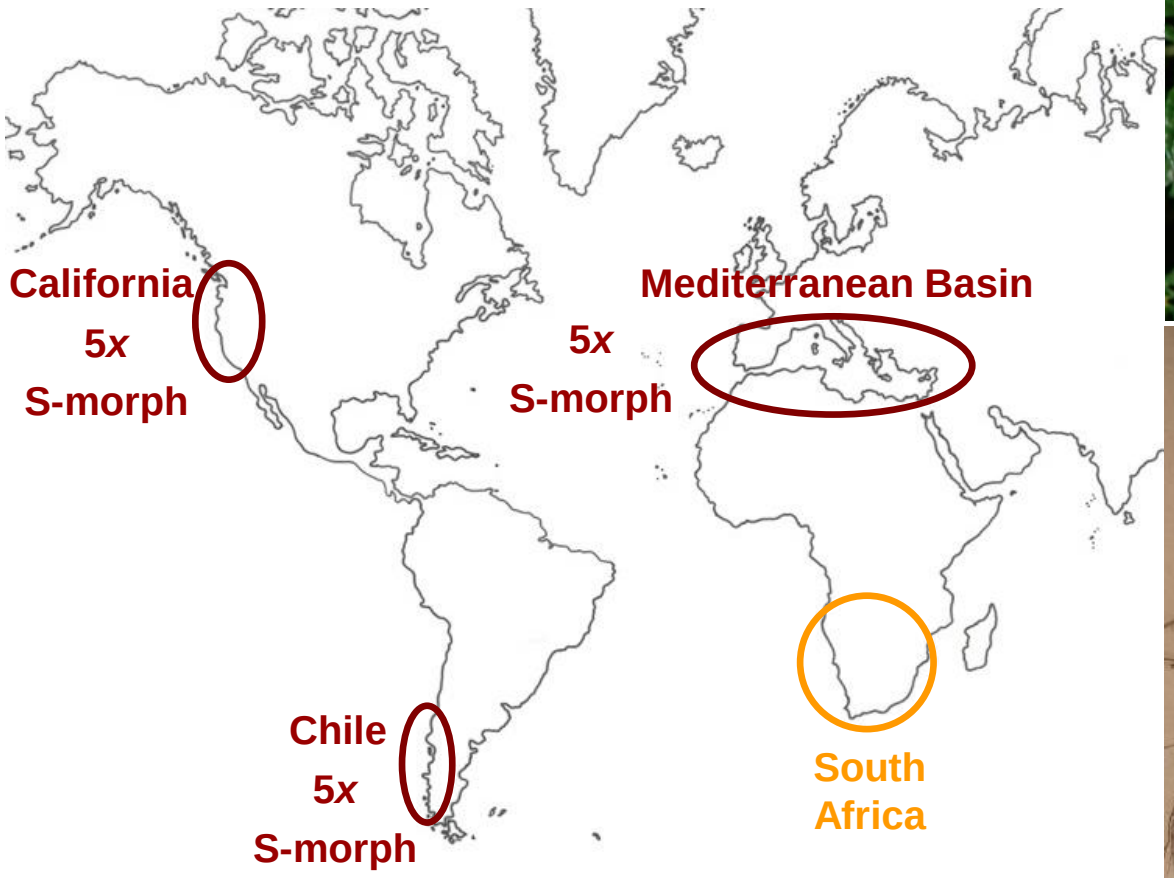
Invaded areas



- Invasive success seems to be highly correlated with anthropogenic activities

Oxalis pes-caprae L.: Distribution patterns and diversity of forms

Invaded areas



Founder events

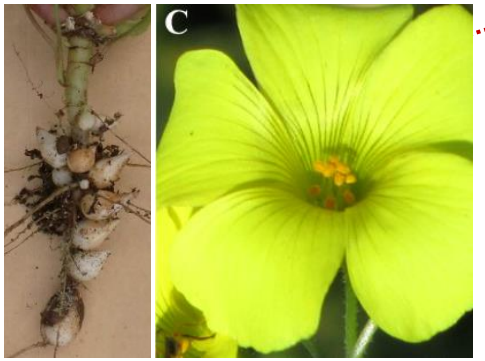
- Clonality clearly played a major role in invasion and enabled growth, persistence and spread after the absence of compatible mating partners.

Bermuda buttercup invasion history: what have we learned so far?

Classic example in biological invasions, known as **a strictly asexual form that successfully spread in Mediterranean** climate regions

Baker 1965

5*x* S-morph



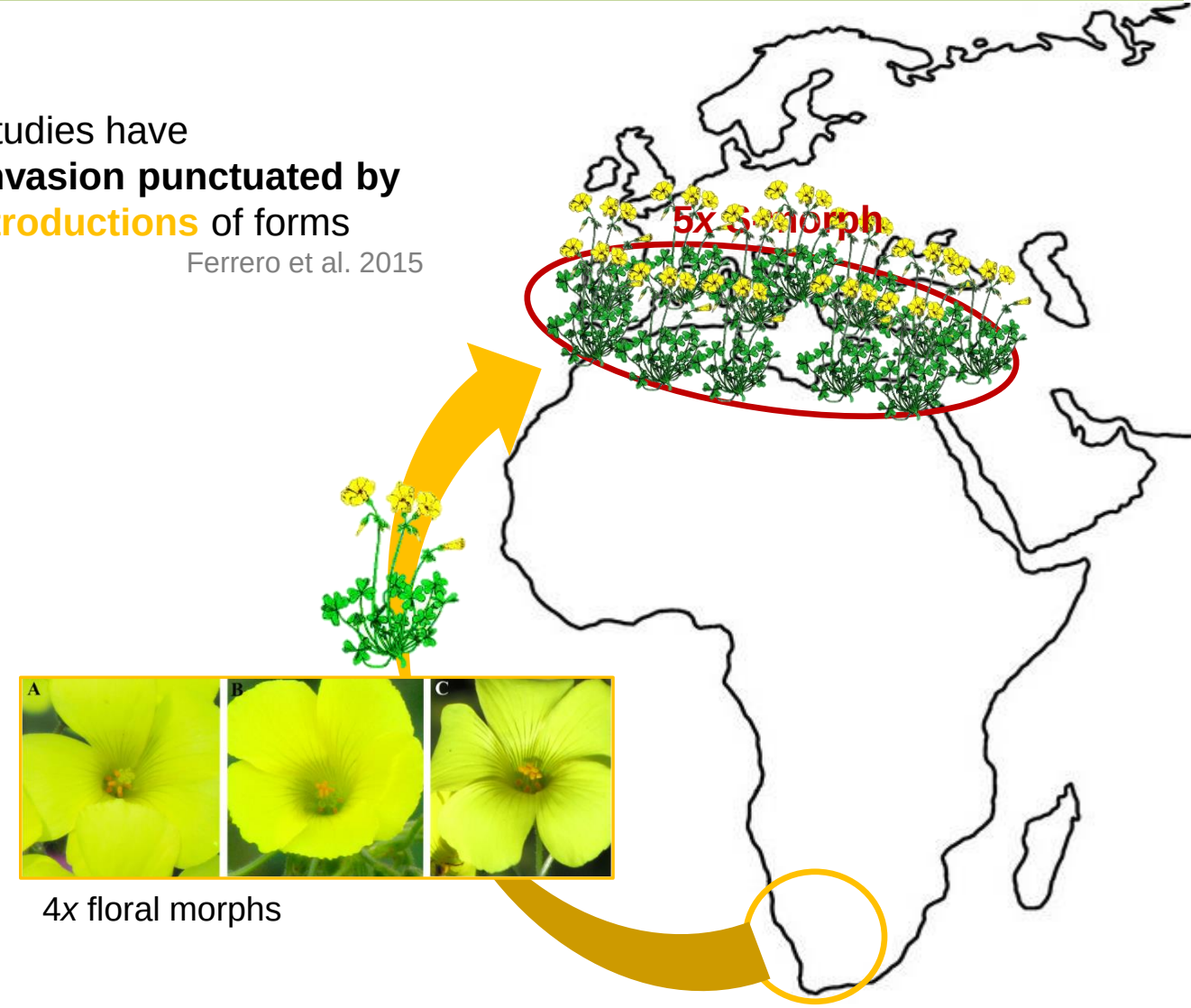
- Incompatibility system
- Lack of compatible mates
- Odd ploidy

Krejciková et al. 2013, Signorini et al. 2013, Ferrero et al. 2015

Bermuda buttercup invasion history: what have we learned so far?

Molecular studies have shown an **invasion punctuated by multiple introductions** of forms

Ferrero et al. 2015

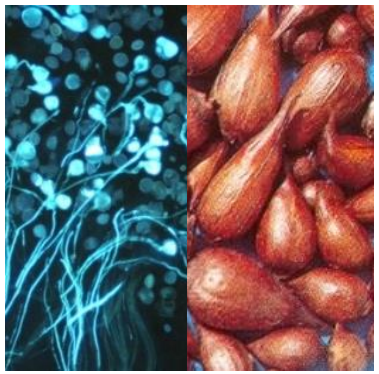


4*x* floral morphs

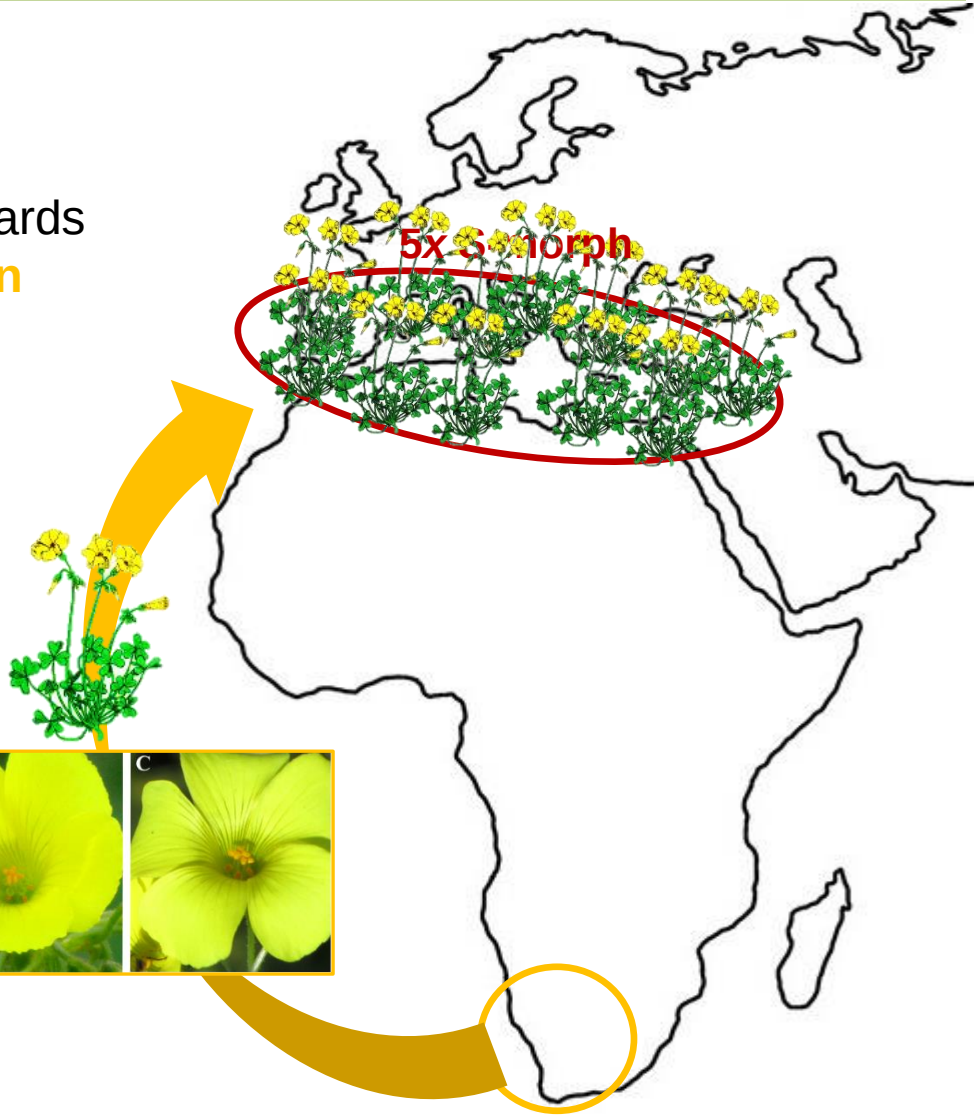
Bermuda buttercup invasion history: what have we learned so far?

Strong **mate limitation**

Selective pressures towards
uniparental reproduction



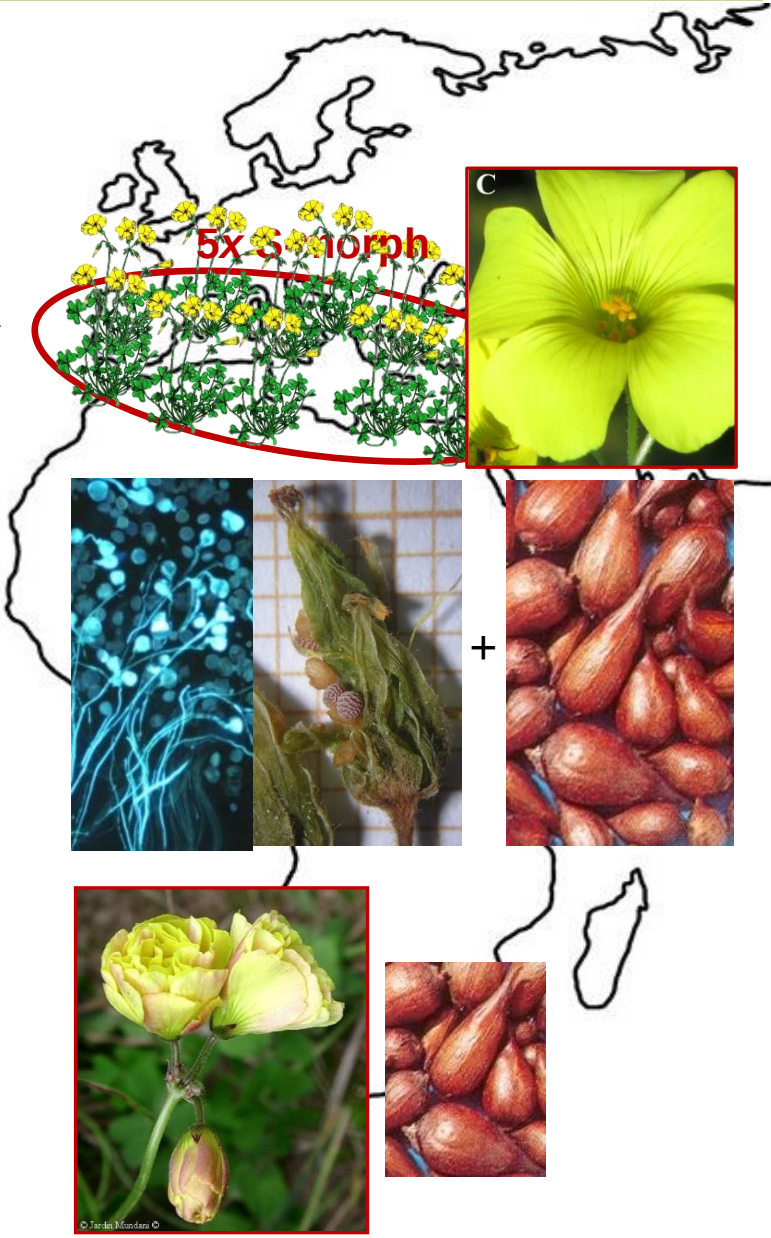
4*x* floral morphs



Bermuda buttercup invasion history: what have we learned so far?

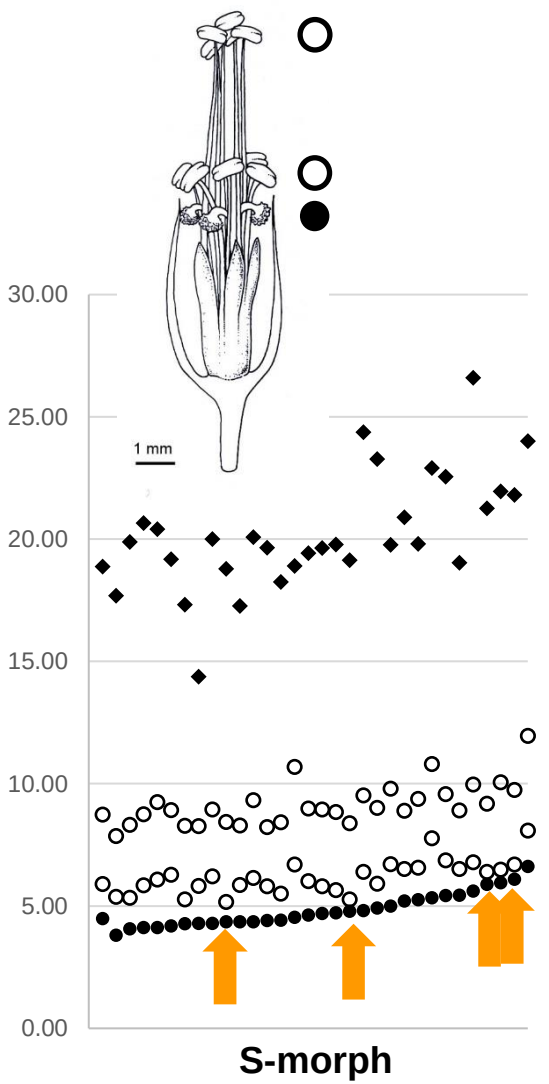
4*x* floral morphs

versus



Bermuda buttercup invasion history: what have we learned so far?

Modifications of the flowers



Bermuda buttercup invasion history: what next?

AIM

Understand the **ubiquity of the changes in sexual strategies** and assess the **impact in the maintenance of flower polymorphisms** by extending the sampling to other regions of the Mediterranean basin

Finding Bermuda buttercup

Finding Bermuda buttercup

CALL FOR COLLABORATION

You or any of your colleagues are **invited to participate** in this project

Finding Bermuda buttercup

PROTOCOL

Finding Bermuda buttercup mates: PROTOCOL

1. POPULATION CHARACTERIZATION

Population information
Floral morph proportions
Database

2. PLANT SAMPLING FOR CYTOTYPE AND FITNESS

Morph voucher
Reproductive fitness samples
Cytotype samples
Genetic samples

3. FLOWER MORPHOMETRY

Flower samples

Finding Bermuda buttercup mates: PROTOCOL

1. POPULATION CHARACTERIZATION

Population information

- Geographical coordinates (WGS84)
- Habitat type (Table 1)
- Crop (if applicable)
- Soil movement
- Notes

Table 1.

Natural or semi-natural areas

Agricultural areas

- Intensive permanent crops
- Traditional permanent crops
- Annual crops

Managed forest areas

Managed urban areas

Non-managed urban areas

Managed peri-urban areas

Non-managed peri-urban areas

Rural areas

Road edges

Other

Example

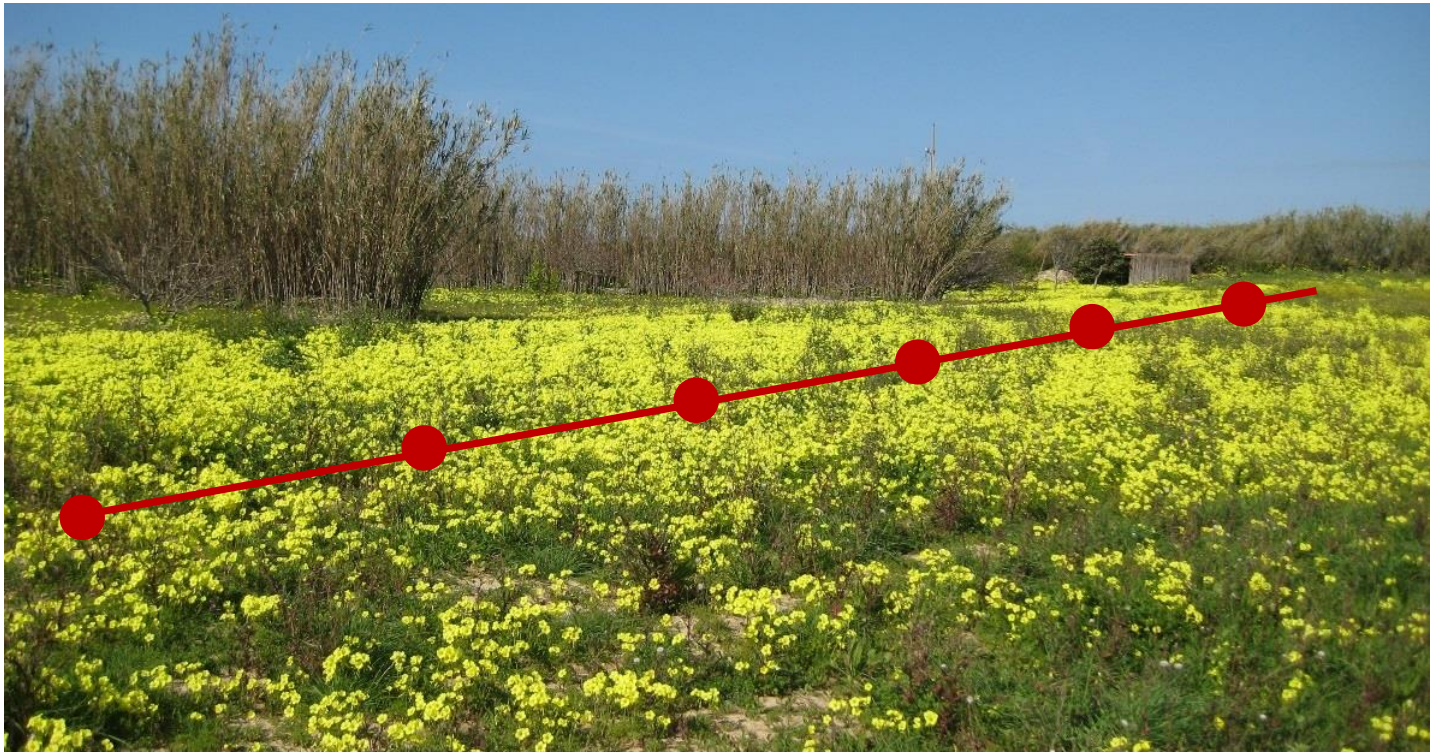
- 39.577621, -9.054498
- Annual crops
- Crop: corn
- Yes
- Notes: plant remnants indicate a corn field

Finding Bermuda buttercup mates: PROTOCOL

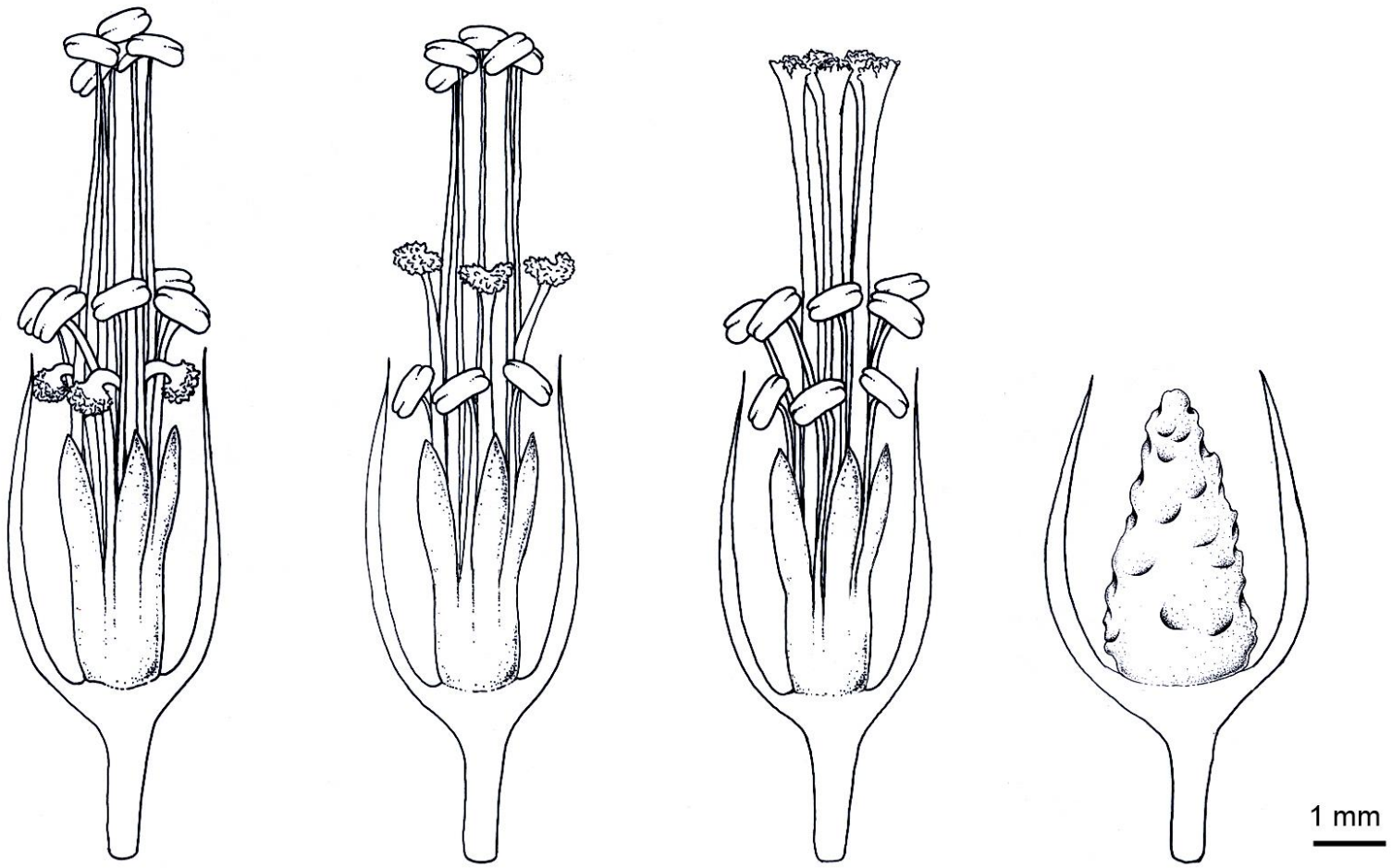
1. POPULATION CHARACTERIZATION

Floral morph proportions

- Observe the morph of at least 100 individuals (in 5-m intervals) across 2-3 longitudinal transects across the entire population

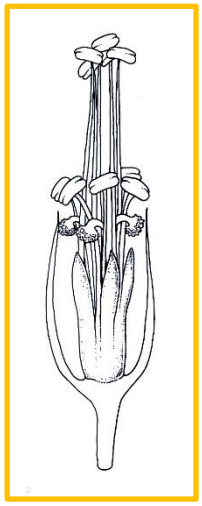


FLORAL MORPHS



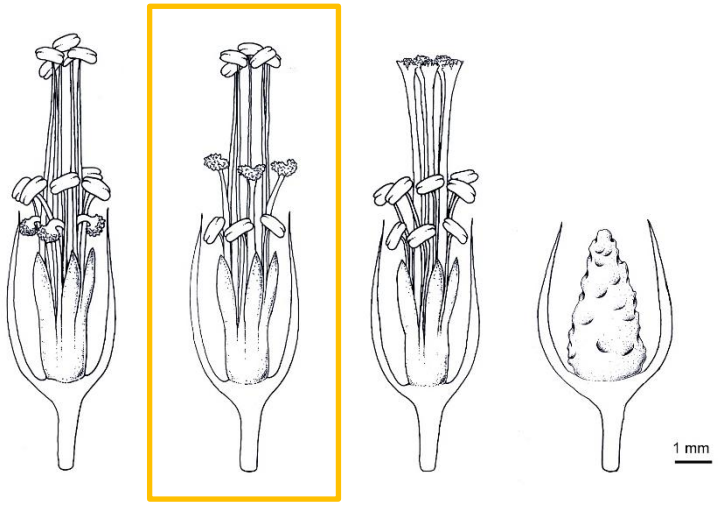
SS **MS** **LS** **St**

FLORAL MORPHS



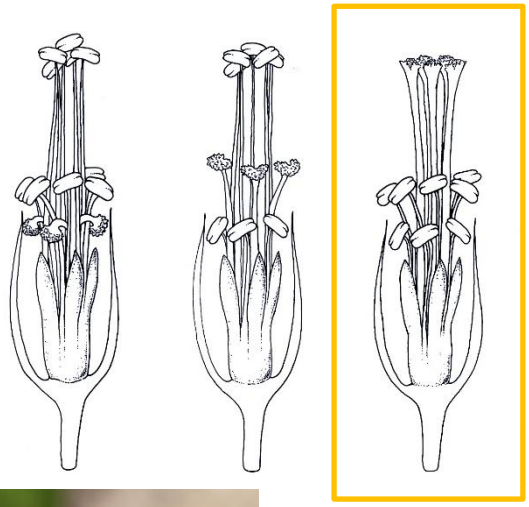
Short-styled morph (SS)

FLORAL MORPHS



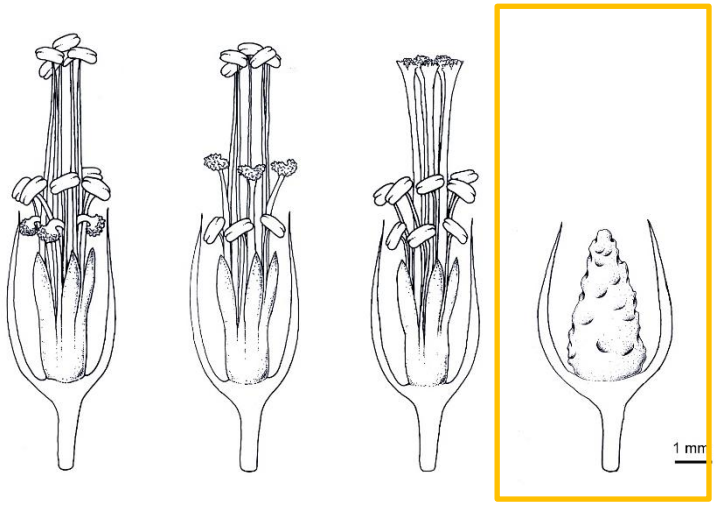
Mid-styled morph (MS)

FLORAL MORPHS



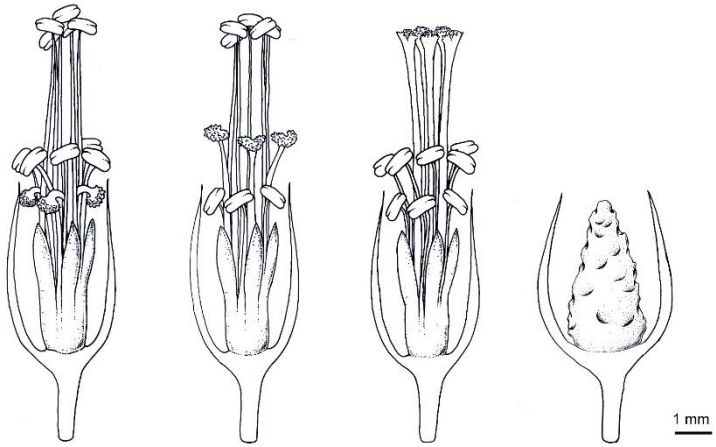
Long-styled morph (LS)

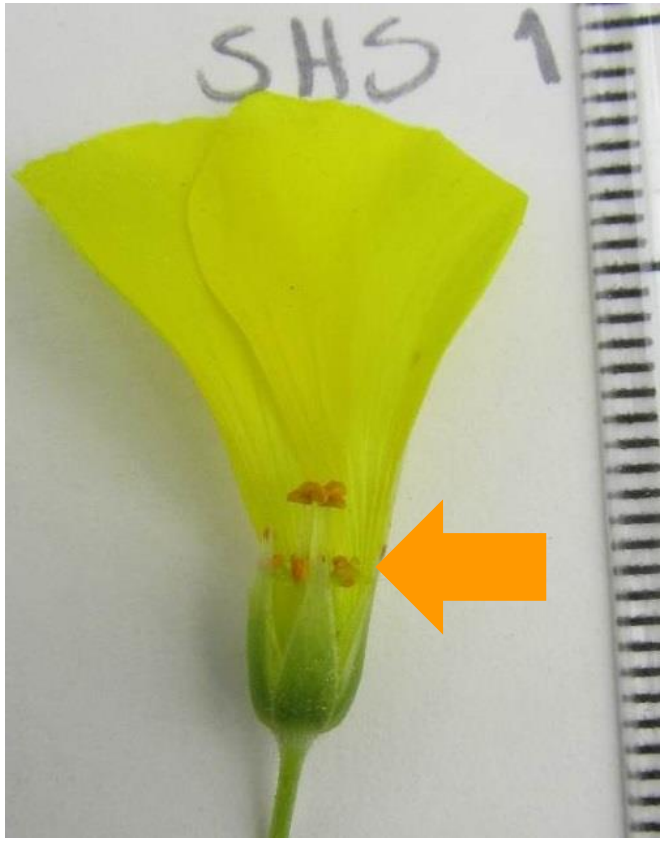
FLORAL MORPHS



Sterile morph (St)

FLORAL MORPHS





Semi-homostylous morph (SHS)

Finding Bermuda buttercup mates: PROTOCOL

1. POPULATION CHARACTERIZATION

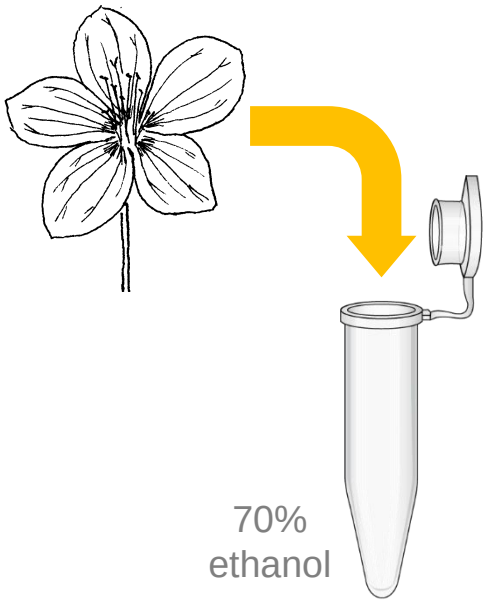
Database: Enter the data in the Excel file provided

	A	B	C	D	E	F
4	**Excel Sheet - POPULATION Database**				**Excel Sheet - FITNESS Database**	
5	**COLUMN NAME**	**DESCRIPTION**			**COLUMN NAME**	**DESCRIPTION**
6	**ID**	Identification code of the locality being sampled. Please use the following coding instructions: Country code_Researcher code_population number_plant number. e.g., PT-SC-25 refers to the population 25 sampled by Sílvia Castro in Portugal.			**ID**	Identification code of the locality being sampled. Please use the following coding instructions: Country code_Researcher code_population number_plant number. e.g., PT-SC-25 refers to the population 25 sampled by Sílvia Castro in Portugal.
7	**Locality**	Lowest administrative unit at the country level where the observations were undertaken			**Locality**	Lowest administrative unit at the country level where the observations were undertaken
8	**Country**	Country where the observations were undertaken			**Country**	Country where the observations were undertaken
9	**Coordinates**	Geographical coordinates provided in World Geodetic System 84 system			**Plant ID**	Number identifiyng the individual plant being sampled
10	**Oxalis coverage (%)**	Aproximate percentage of Oxalis pes-caprae coverage in the locality			**Morph**	Floral morph of the individual plant being sampled
11	**Population area (m^2)**	Estimated area occupied by the population in square meters. If the population is a continuous in the region please indicate 'continuum' in column 'Notes'			**No. flower buds**	Number of flower buds in the selected flowering/fruting stem
12	**Habitat**	Characterize the habitat in the following categories: (Table 1 and Excel Sheet "Table 1 HABITAT"). Any additional information can be provided in the field "Notes"			**No. flowers**	Number of open flowers (including recently open flowers to wiltered flowers) in the selected flowering/fruting stem
13	**Crop**	If the population is in agricultural area please indicate the crop			**No. fruits**	Number of developed fruits in the selected flowering/fruting stem
14	**Soil movement**	YES/NO and should refer to the occurence of soild movement during the last year			**No. scars**	Number of scars corresponding to the number of aborted flowers (i.e., flowers that did not developed into fruit) in the selected flowering/fruting stem
15	**Co-flowering plants**	List co-flowering plant species present at the locality				
16	**Floral forms**	Number of plants of each floral form observed in the transects across the population. Floral forms:				
17		SS - Short-styled				
18		MS - Mid-Styled				
19		LS - Long-styled				
20		SHS - Semi-homostylous				
21		St - multipetalous sterile form				
22	**Notes**	Please use this space to add any note you may find pertinent				

2. PLANT SAMPLING FOR CYTOTYPE AND FITNESS

Locate 10 plants from each morph and collect the following samples in every plant:

- Morph voucher: collect 1-2 flowers per plant to separate microtubes with ethanol 70% (1 microtube per plant) labelled inside with population and plant codes



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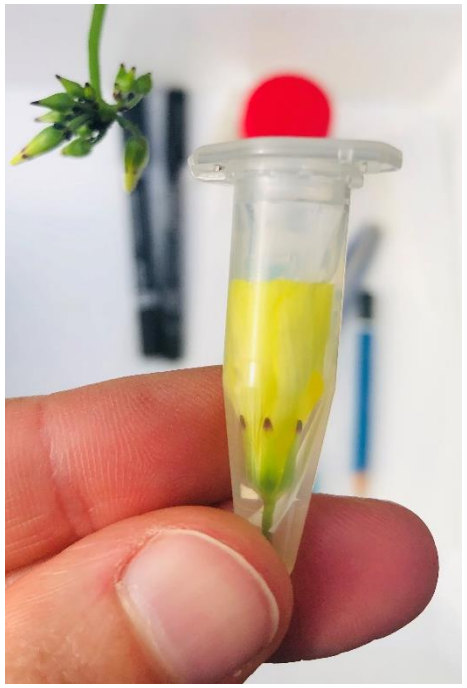


2. PLANT SAMPLING FOR CYTOTYPE AND FITNESS

Locate 10 plants from each morph and collect the following samples in every plant:

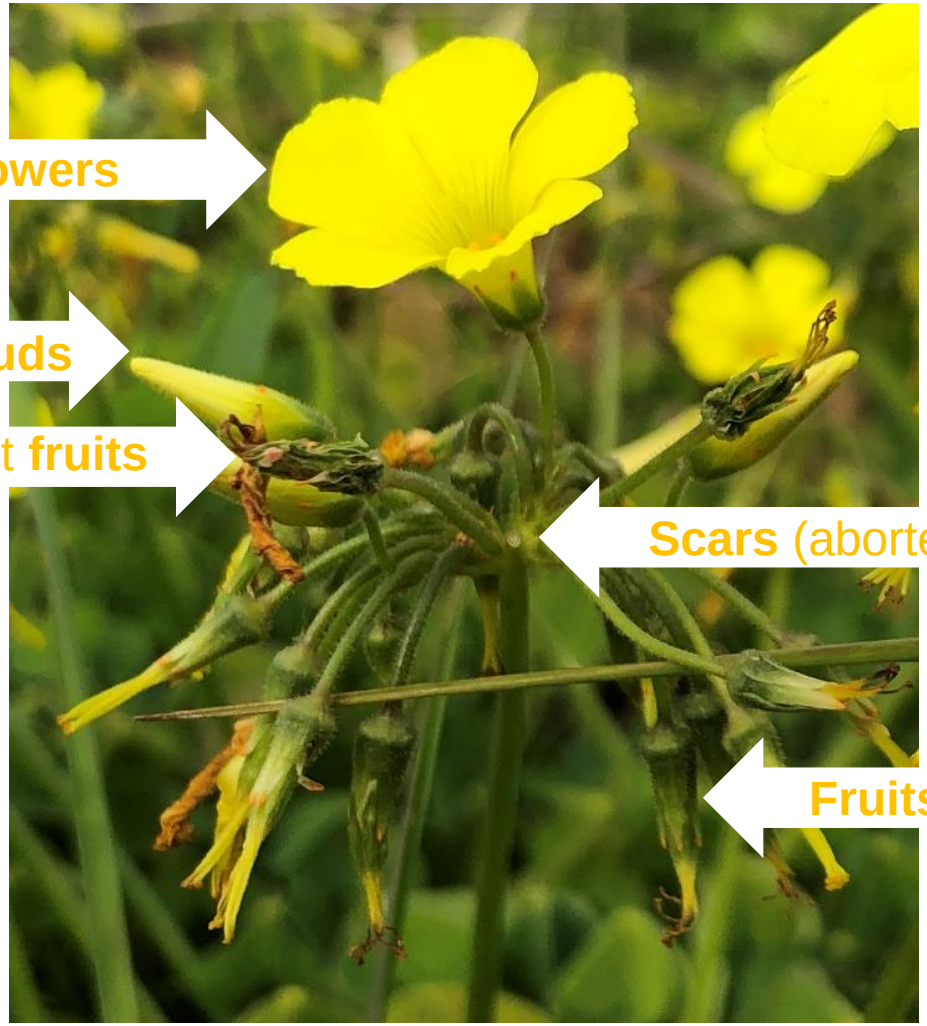
- Reproductive fitness: you can proceed in one of two ways

1) identify a fruiting stem per plant and count the number of scars (i.e., aborted flowers), fruits, flowers and flower buds (if applicable); collect **3 non-dehiscent fruits** (when available) into a paper bag identified with the population and plant codes;
OR
2) collect a fruiting stem per plant into a paper bag identified with the population and plant codes.

Notes: it is likely that fruits do not exist, thus, look for inflorescences with scars indicative of zero fruits

Place the bags to dry at 30 ºC until completely dried.

SEXUAL REPRODUCTIVE FITNESS



SEXUAL REPRODUCTIVE FITNESS

Fruits (after dried in paper envelopes)

Fruits (non-dehiscent)

2. PLANT SAMPLING FOR CYTOTYPE AND FITNESS

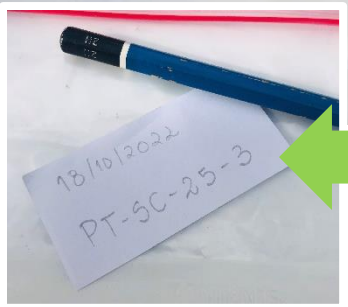
Locate 10 plants per morph and collect the following samples in every plant:

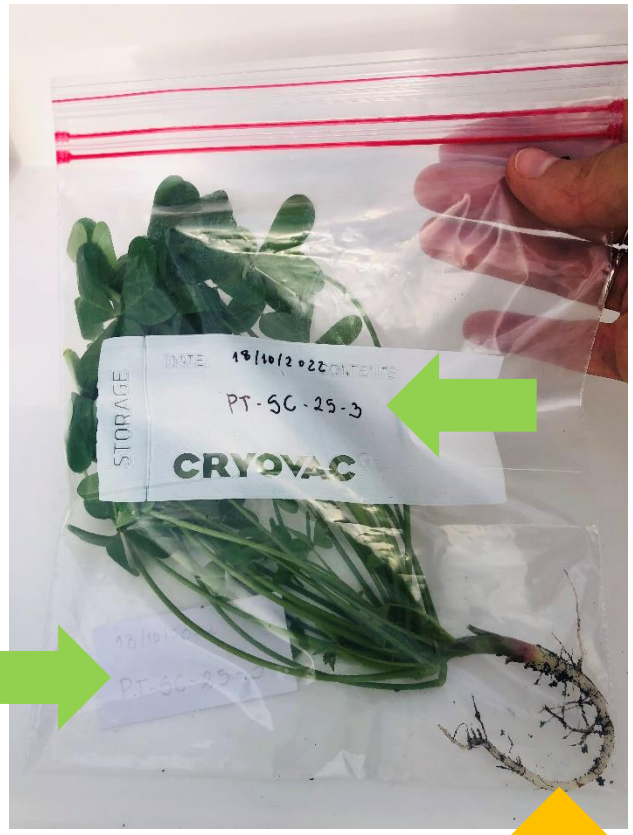
- Cytotype analyses: collect the plant with some root system, prune the old leaves, store it in a small plastic bag identified with population and plant codes and stored at 4 ºC

IMPORTANT: fresh samples need to be **sent as fast** as possible for flow cytometric analyses to:

Sílvia Castro
Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

SAMPLES FOR FLOW CYTOMETRY





Root system

Label

2. PLANT SAMPLING FOR CYTOTYPE AND FITNESS

Locate 10 plants per morph and collect the following samples in every plant:

- Genetic analyses: collect 4-6 young and healthy leaves per plant and store in separate paper envelopes with silica gel, labelled with population and plant codes

Notes: all the sampled plants should be separated at least 5-m apart to avoid re-sampling clones

3. FLOWER MORPHOMETRY

- Collect at least **25 flowers** from each morph to a vial with 70% ethanol labelled inside with population and plant codes
- Different morphs should be stored in separate vials

Send the flower samples to:

Peter Glasnović

University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, University Campus Livade, Livade 4, 6310 Izola, Slovenia

Finding Bermuda buttercup mates: PROTOCOL

POPULATION CODING

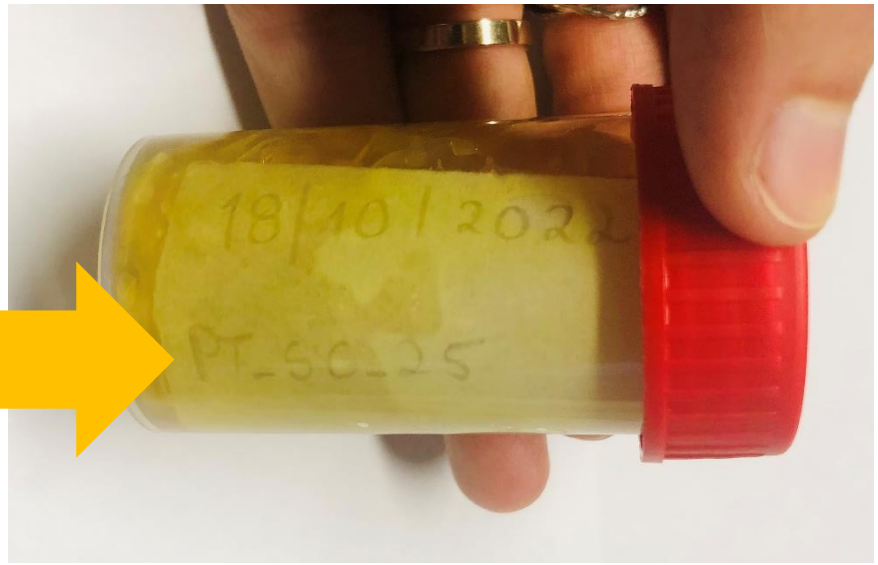
Country code_Researcher code_population number

e.g., **PT-SC-25** refers to the population 25 sampled by Sílvia Castro in Portugal.

PLANT CODING

Country code_Researcher code_population number_plant number

e.g., **PT-SC-25-03** refers to the plant number 3 from the population 25 sampled by Sílvia Castro in Portugal.



Finding Bermuda buttercup mates: PROTOCOL

NOTE: flowering period may be different in your region

TIMELINE

2022	Oct	•	WG1 Meeting to **present** the project
	Nov	•	Development of the **final field protocol**
		•	**Participants manifestation of interest (11 Nov)**
	Dec	•	**Workshop** for protocol demonstration and questions
2023	Jan	•	Sample collection (Dec 2022 to Mar 2023)
	Fev	•	FCM analyses (Dec 2022 to Mar 2023)
	Mar		
	Apr	•	Flower analyses in the lab (Jan-Jul 2023)
	May		
	Jun	•	Preliminary data analyses (Jun-Jul 2023)
	Jul		
	Ago	•	**Presentation of results** and next steps (MC Meeting)

FLOWERING

Bermuda buttercup invasion history: what next?

CALL FOR COLLABORATION

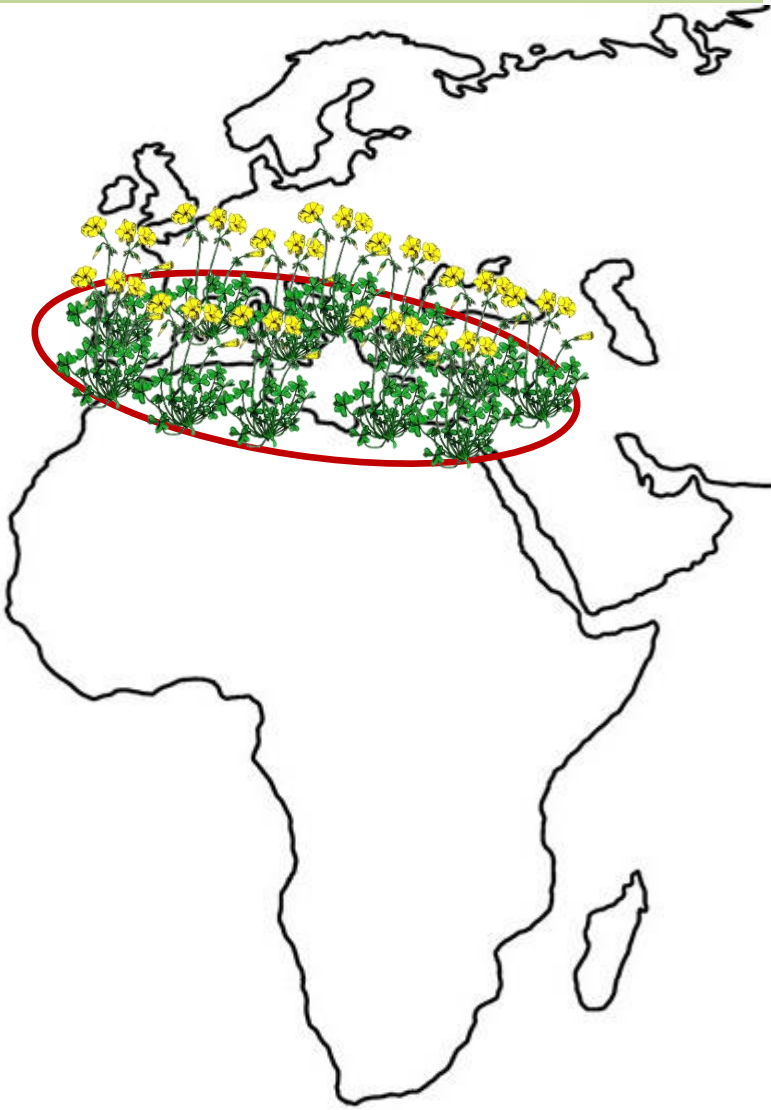
You or any of your colleagues are **invited to participate** in this project

MANIFESTATION OF INTEREST until 11th November

OUTPUTS

Participants will be included as **co-authors** in the **manuscript** describing **current patterns in the Mediterranean basin**

Additionally, participants can be included in further works upon their involvedness in the project



QUESTION?

Looking forward for your participation!

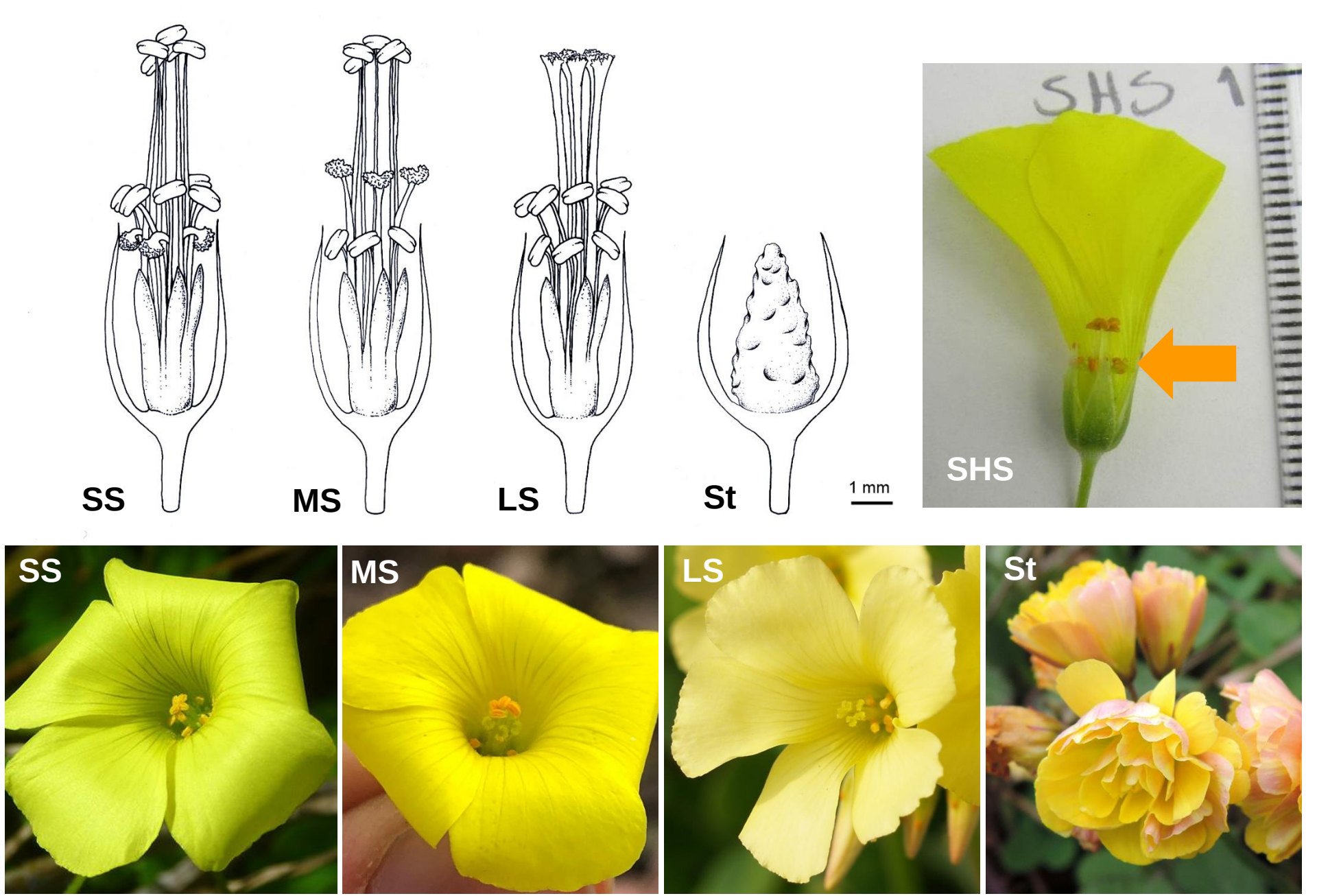


Figure 1. Bermuda buttercup (*Oxalis pes-caprae*) floral forms: short- (SS), mid- (MS) and long-styled (LS) morphs, multipedal sterile form (St) and semi-homostylous morph (SHS).