



HOW TO SAVE A SELF-INCOMPATIBLE SPECIES FROM A UNIQUE PURE SPECIMEN FOUND WORLDWIDE: THE CASE OF THE CARTAGENA'S ROCKROSE.

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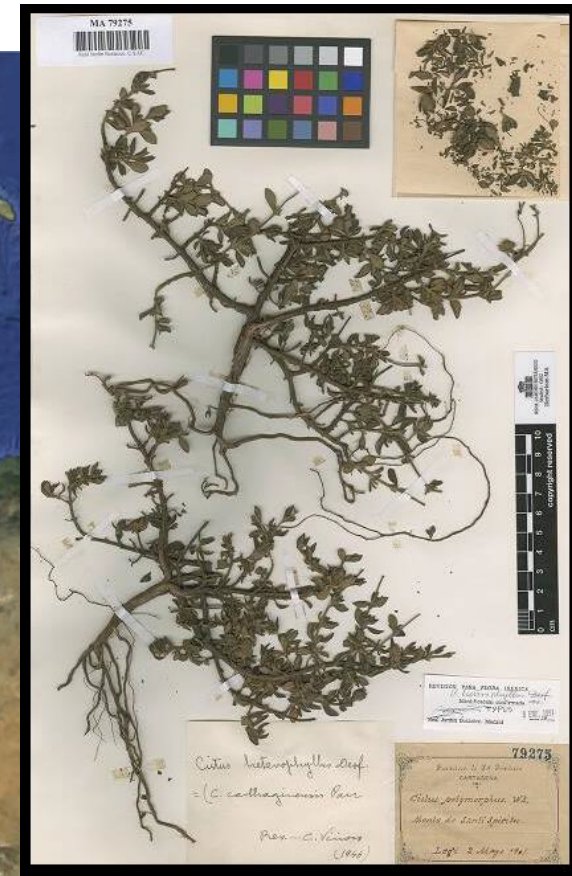
Cistus heterophyllus

A rockrose of Northern Africa distributed from Morocco to Algeria, having a disjunctive distribution area in E and SE Spain –through subsp. *carthaginensis*–.



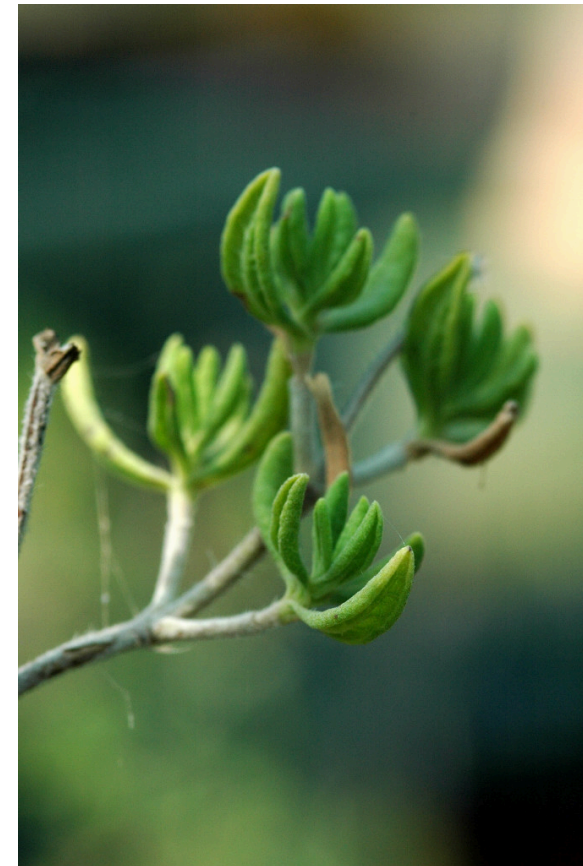
Cistus heterophyllus* subsp. *carthagenensis

First found in Carthage (Region of Murcia) in 1901 and described as *Cistus carthagenensis* in 1904



A theoretically extinct species

It was considered extinct in Cartagena mountains since mid XXth century



Found in Pobla de Vallbona (Valencian Community) more than 300 km North but only one specimen!

Only one individual, found in 1986. No new one has been found afterwards, and its vigor has been declining progressively

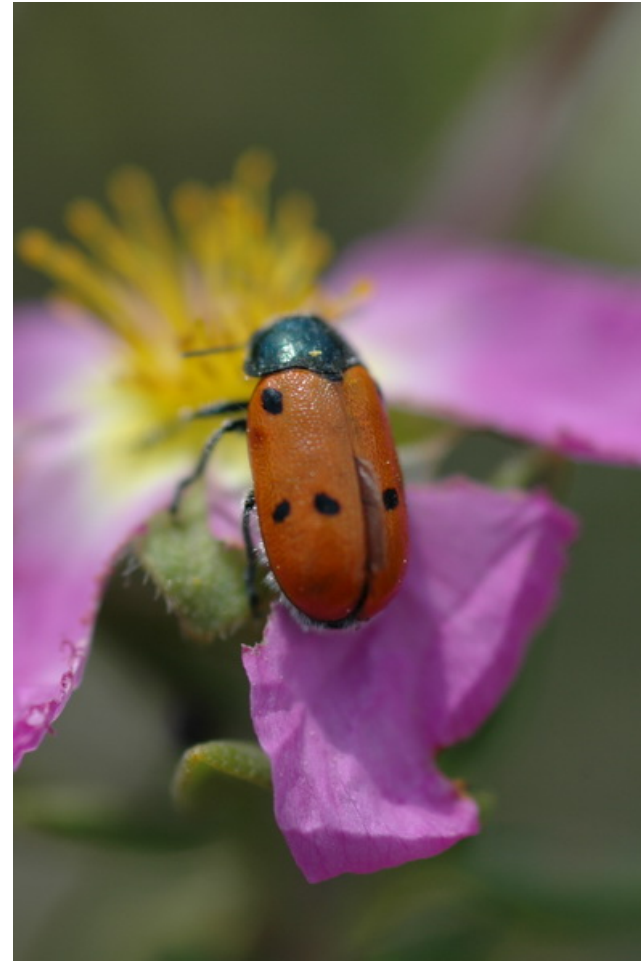
In 1988, the name was recombined, considering that the re-discovered Spanish plants should be considered only a subspecies: *C. heterophyllus* subsp. *carthaginensis*



The main problem: How to obtain new plants?

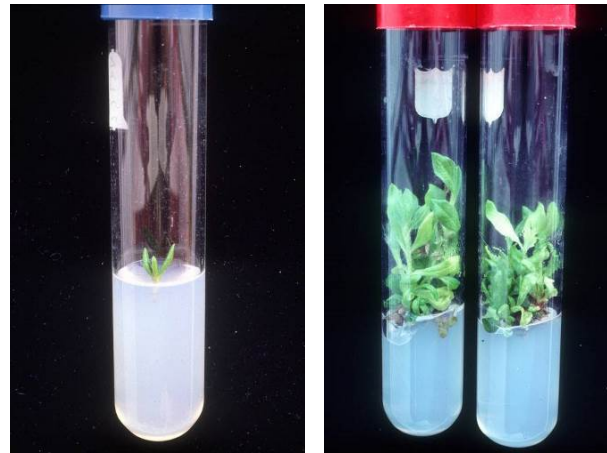
As most *Cistus* species, *C. heterophyllus* subsp. *carthaginensis* is self-incompatible. Thus, having only 1 individual, seed production should not to be expected.

Actually, the plant produced hundreds of flowers and the young fruits fell before maturing, but 0-2 fruits bearing 1-2 non-viable seeds were produced most years. Additionally, several insects caused damages to flowers and young fruits, but even protecting the plant against their attacks no new viable seeds were found.



The apparent good solution

In 1990, the Valencian Institute of Agronomic Research (IVIA) obtained a successful protocol for *in vitro* propagation, and dozens of new clonal plants were produced.



Planting the new clonal plants.

In 1991 the IVIA made a first plantation in an open, dry pinewood (*Pinus halepensis*) in their own enclosure.

In 1997, several plantations were made in natural sites near Poble de Vallbona. Only one plantation of 25 clonal individuals on the site ‘Tancat de Portaceli’ survived, and in fact is currently alive -6 plants in 2018, 24%-.

This site Tancat de Portaceli was declared Plant Micro-reserve (PMR).



A little hope....

Between 1997 and 2010, up to 8 plantations were made, but only this of PMR Tancat de Portaceli survived and yielded some new plants.

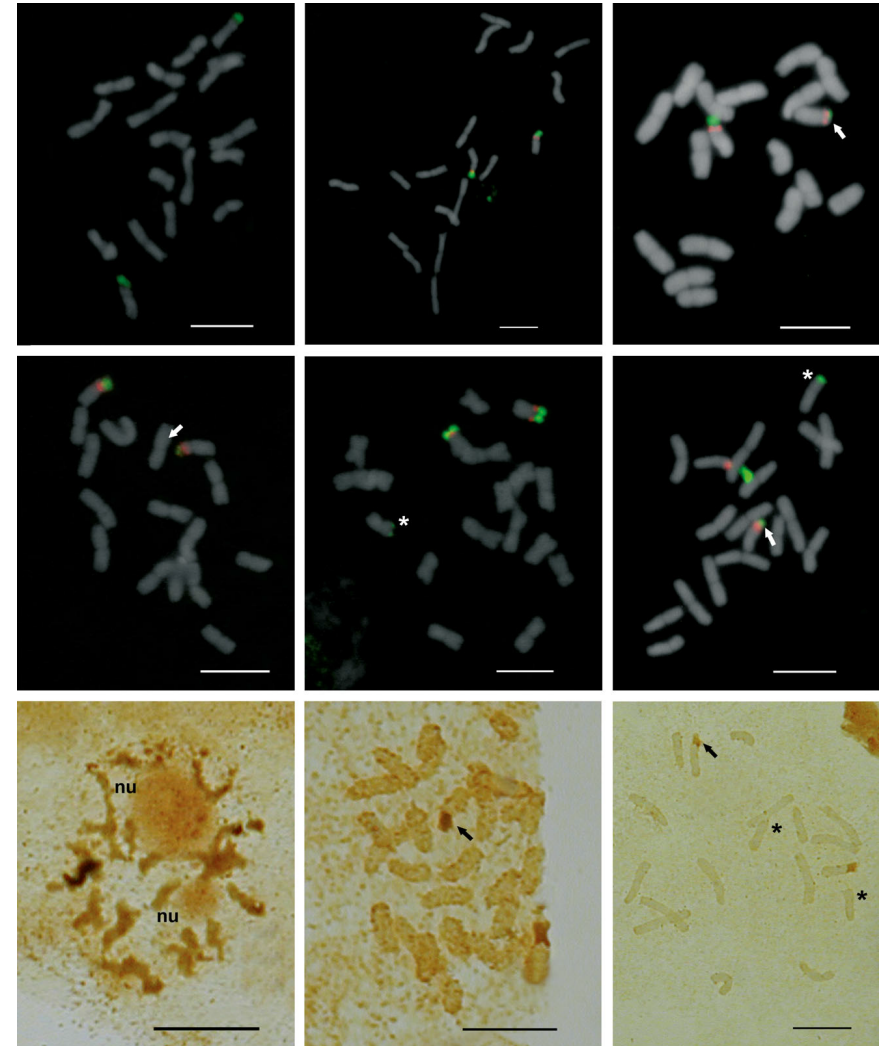
New plants found there, obtained after sexual propagation, belonging mostly to the hybrid *C. albidus* x *C. heterophyllus* subsp. *carthaginensis* (*C. x clausonis* nothosubsp. *crespoi*). In 2012 two small plants apparently pure -without hybridization- were found, but they did not support the strong natural drought in 2014



... but a volatile hope

Works developed by researchers of the Botanical Garden of the University of Valencia after 2013, demonstrated that the *in vitro* procedures to obtain new plants, also produced as collateral damage a change in the fluorescence band, in the chromosomal region of rDNA (just the band affecting the development minor subunit of the ribosome).

Although that mutation perhaps could not be coding, the *in vitro* way should be preventively abandoned.



... and a serious enemy

The work in Tancat de Portaceli indicated that the main enemy for the conservation of this species was the hybridization with *Cistus albidus*. Any new area to plant *C. heterophyllus* sh



New plants in the Region of Murcia

In 1993-1994 two small populations were discovered in Cartagena (Murcia), close to the original site where the plant was found in 1901.

Both populations were burned by a forest wildfire in 1998 and only one (Llano del Beal) survived, recruiting from the soil seed bank.

As demonstrated by molecular analyses the Murcian plants never have some degree of hybridization, although a few of them can be considered extremely close to pure individuals.

At least apparently, the unique (sure) pure plant of *C. heterophyllus* subsp. *carthaginensis* is the extant one in Puebla de Vallbona (Valencia).



Searching for a solution.

Along the early 2010s several actions were planified:

-Artificial hybridizations ex situ under controlled conditions, crossing *C. heterophyllus* subsp. *carthaginensis* (from Valencia), *C. x clausonii* nothosubsp. *crepoi* (from Murcia) and *C. heterophyllus* subsp. *heterophyllus* (from Morocco). All the crosses were successful and giving highly viable hybrid seeds. Seeds were stocked for future scientific research (to serve to research centers if needed).

-Obtaining of new clonal plants, but only from cuttings directly obtained fro the mother plant in Pobla de Vallbona.

-Further artificial pollinations between the new clonal plants (without in vitro procedures) as well as transferring pollen to/from the same mother plant in field.

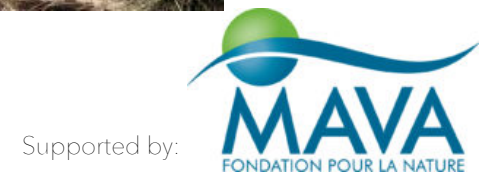


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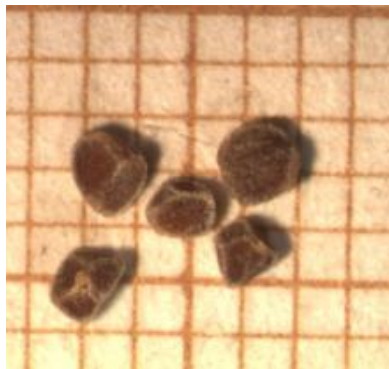
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An apparent but non-viable way.

In 2012, a fruit with very few seeds was collected on the mother plant in Pobla de Vallbona. 2 seeds produced new plants; only one of them survived, but giving a teratological individual.



A slot in the self-incompatibility.

During 2013 the self-incompatibility in the mother plant in Pobla de Vallbona was slightly broken because of unknown reasons. Close to 50 seeds were collected.

In 2014 two lines of Valencian plant material were obtained, both from the unique pure wild plant:

- from the cuttings (before indicated)
- from seeds



Working with the pure new plants .

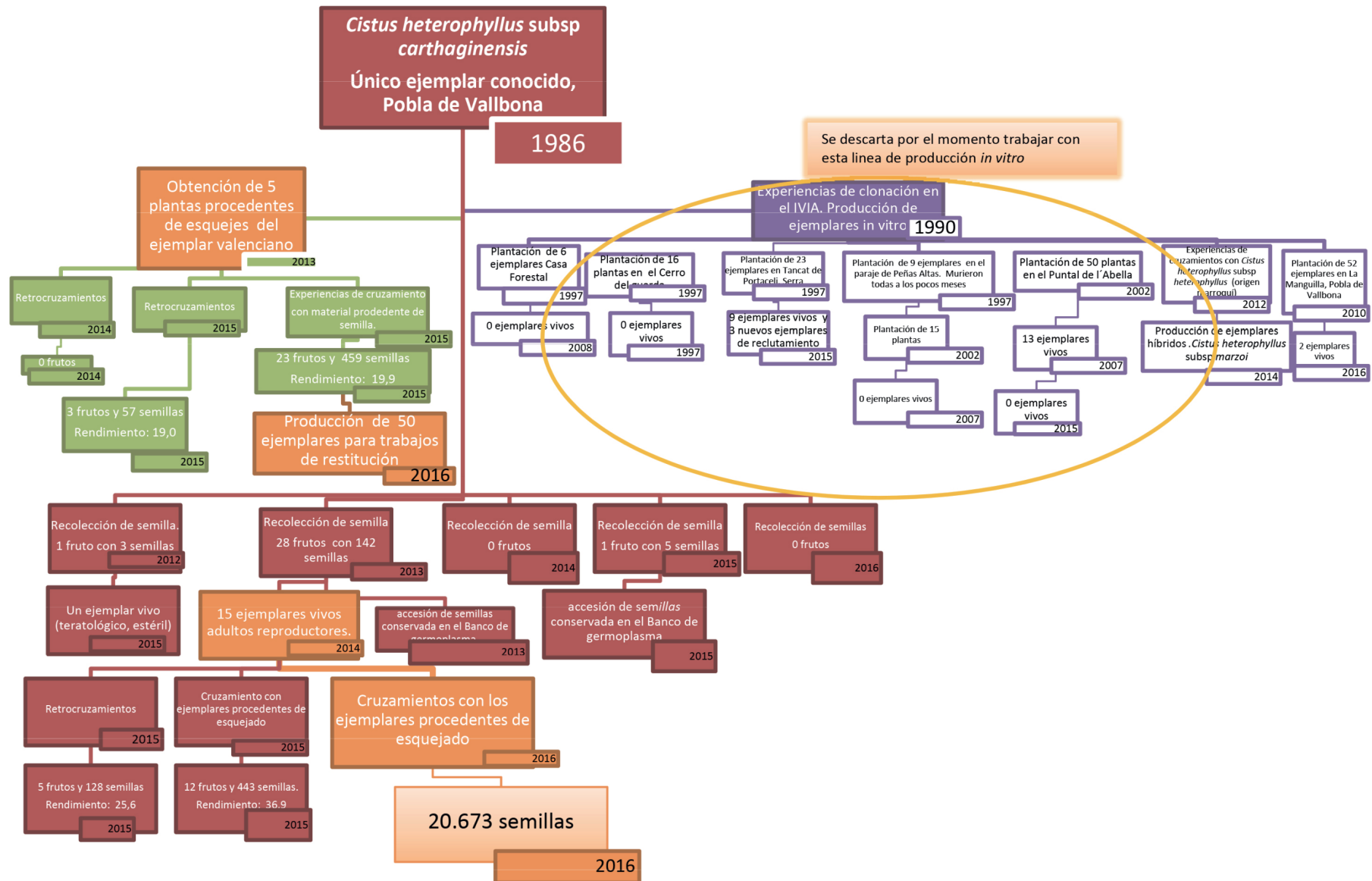
Plants obtained from seeds and cuttings grown in 2014-2015, reaching the fertile age in 2016.

Artificial crosses were made, making all the combinations of donor/receptor of pollen between origins (seed and cuttings), as well as using the mother plant in field. The best combination found was using plants from cuttings as pollen receptors, and coming from seed as donors



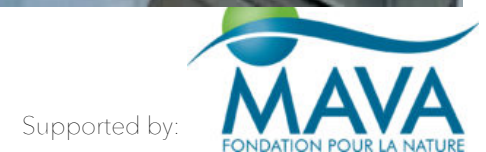
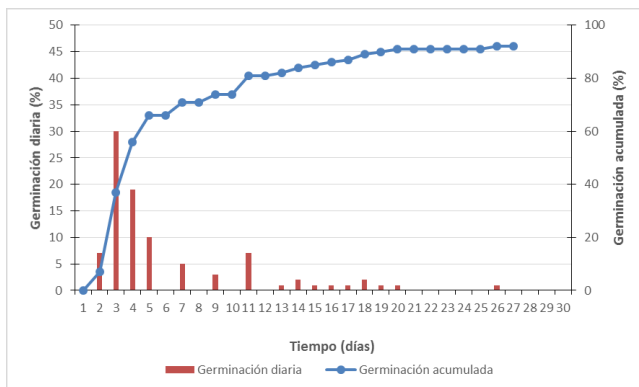
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Working with the pure new plants .

In 2016, 20.673 seeds of pure Valencian plants were obtained



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Following the right path

Artificial crosses were also made in 2017, obtaining 18.663 seeds. Viability is over 85%

In 2018 pollination was made using bumblebees and the performance obtained (amount of seeds per plant) was 1.398 seeds/plant, bigger than obtained through artificial pollination (maximum average 1.034 seeds/plant). 76.890 seeds were obtained.

Year	Pollination time (days)	Nr. of pollinated plants	Time spent in manual pollination (h)	Fruit formation				Nr of seeds	Performance (seeds per fruit)
				Nr of fruits not set	Nr of fruits set	Total nr of fruits	Successful fructification (%)		
2015	33	14	630	190	43	233	18,44	1.087	25,3
2016	43	20	1.935	1.105	550	1.655	33,23	20.673	37,6
2017	42	33	2.375	911	881	1.792	49,16	18.663	21,2
2018	49	55	0	1.254	2.091	4.145	69,75	76.890	36,8



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Producing and planting new plants.

Massive production (over 1000 plants/year) started in 2016.

Since December 2016, 1.242 plants have been planted in 8 sites belonging to the Generalitat Valenciana, City councils and the Ministry of Defence. Some plantations involved local people and schools.

The first 25 individuals planted in 2016 produced flowers and viable seeds in 2017 and 2018. We were waiting for new individuals produced in situ.



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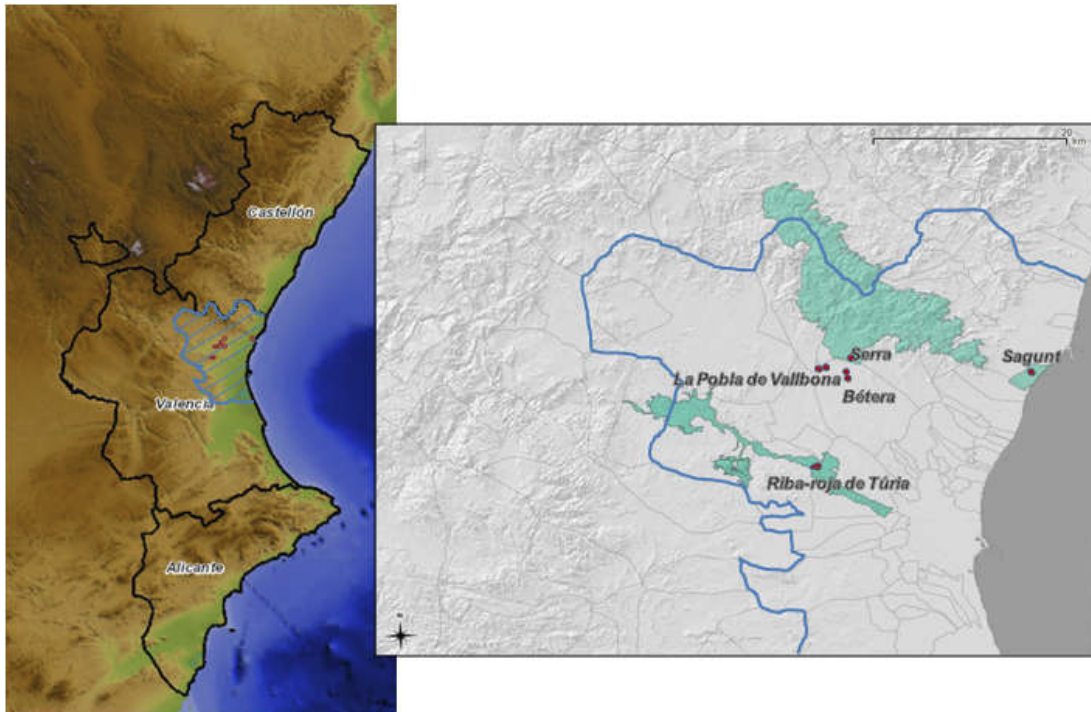
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Cómo salvar del
PELIGRO DE EXTINCIÓN
a la
Jara de Cartagena
— *Cistus heterophyllus subsp. carthagenensis* —



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AJUNTAMENT DE LA POBLA DE VALLEBONA
Ajuntament de Bellera
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Many thanks for your attention



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