















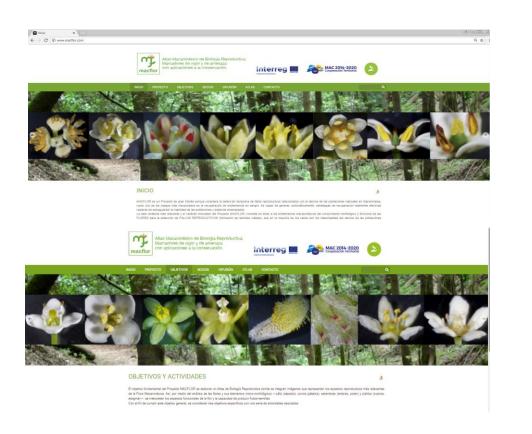


Website of MACFLOR Project

Febles R.¹, Fernández-Palacios O.¹, Olangua-Corral M.¹, Roca Salinas, A.², Casimiro, P.G.P.³, Freitas, C.F.³, Gouevia, M.⁴ & Sequeira, M.⁴ & Pérez de Paz J.¹ ¹ Dpto Biología Reproductiva y Micro-Morfología & ²Banco de Germoplasma, Jardín Botánico Canario "Viera y Clavijo" JBCVC. Cabildo de Gran Canaria. E-mail: <u>rfebles13@gmail.com</u>. ³ Jardim Botânico do Faial. pedro.gp.casimiro@azores.gov.pt catia.f.freitas@azores.gov.pt. ⁴ Universidade da Madeira mgouveia@uma.pt sequeira@uma.pt

The website of MACFLOR Project is presented: http://www.macflor.com

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Flower.

Micro-morphology

and formation of

fruits

CHOICE OF ENDEMISMS

(according to sexual systems and self-incompatibility)

FLOWERS & SEXUAL SYSTEMS

(characterization)

Natural populations

(characterization,

seasonal phenology

and sampling)

Choice of species

and populations.

Bibliographic

background



FLORAL BIOLOGY & PHENOLOGY

BREEDING SYSTEMS. AUTO-INCOMPATIBILITY

(detection)

Dichogamy.

Pollination & floral visitors

Neochamaelea.

Paternity analysis. Pollen gene flow

between sexual phenotypes

Autogamy & xenogamy rate.

Self-incompatibility (detection)

Andryala.

Genetic diversity of genus in

Madeira

This Project involves the Jardín Botánico Canario "Viera y Clavijo"-ua CSIC (Cabildo de Gran Canaria), which acts as leader, the Jardim Botânico do Faial (AZORINA Sociedade de Gestão Ambiental e Conservação da Natureza SA, Direção Regional do Ambiente, Azores) and the Universidade da Madeira. The project has a duration of four years (2017-2020).

OBJECTIVES & ACTIVITIES

The general and main Objective of MACFLOR "Macaronesian Atlas of Reproductive Biology" is to develop the most relevant reproductive traits of the Macaronesian Flora.

This involves the analysis Morphological and Functional of the Flowers and their elements -calyx (sepals), corolla (petals), androecium (stamens, pollen) and gynoecium (pistils, ovules)- to know its capacity to produce fruits and seeds. This knowledge allows to detect **Reproductive Failures** and so to design **Effective Recovery Strategies** of endangered species.

This general objective considers three partial specific objectives and a series of associated activities:

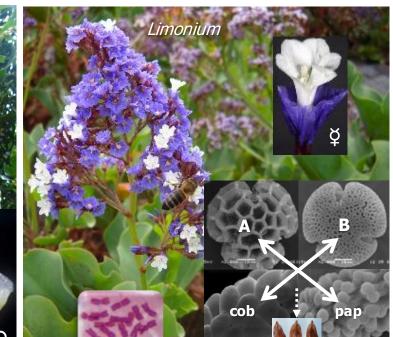
MACFLOR

Macaronesian Atlas of Reproductive Biology

The **FIRST PARTIAL OBJECTIVE** include:

THE CHOICE OF TAXA & POPULATIONS TO STUDY

Principally, Macaronesian endemic plants with **Sexual** polymorphisms and Self-incompatibility Systems were selected, because these reproductive syndromes are more susceptible to reproductive failure. Populations in Sites of Natura 2000 Network, Laurel forest ecosystems and/or Biosphere Reserves are priorities.



Bibliographic background

Highlight the activity aimed at the exhaustive search of bibliographic antecedents. In addition to basic references on reproductive biology, original descriptions, chorology, etc., it is important to review the molecular phylogenies due to the predictive character of the evolutionary history of the morphological and physiological traits of the flower.

NATURAL POPULATIONS & REPRODUCTIVE SAMPLING

To characterization each population, data of location (UTM coordinates, altitude), orography, size, etc, are checked. The seasonal phenology (flowering & fructification) is determined and reproductive sampling (inflorescences, buds, flowers, fruits and seeds) and herbarium sheet are carried out. The different sexual (hermaphrodite or unisexual flowers: & & ♀) or incompatibility phenotypes (pin-thrum, pap-cob) are identified and the sexes or morphs ratio evaluated.



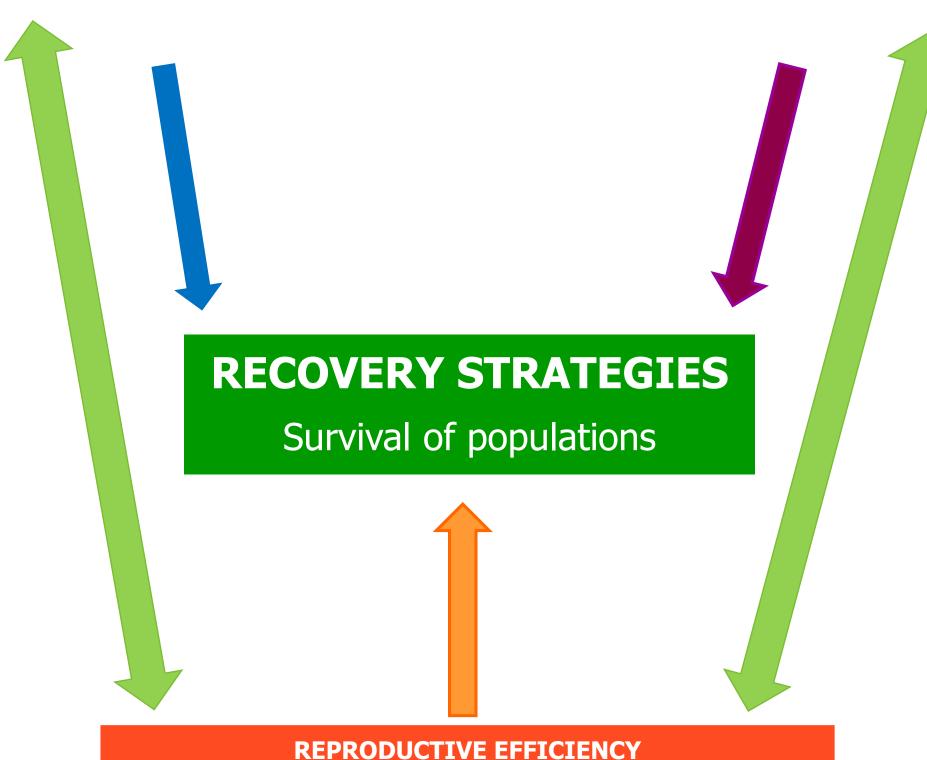
he collected reproductive naterial is stored in a freezer and/or in 70% ethanol (inflorescences, buds & flowers) or in cellulose bags for palynological studies and fruits & seeds for eproductive success studies). Finally the seeds are stored in Seed Banks of the participating institutions for their conservation.



FLOWERS, FRUITS & SEEDS. MICRO-MORPHOLOGICAL CHARACTERIZATION. LABORATORY

Different **preparations** of the floral whorls, as well as fruits and seeds, are elaborated for their observation and capture of images in:





(formation of fruits and viable seeds according to reproductive phenotypes)

Pre-emergent reproductive success Post-emergente reproductive success (Fruit/Flor ratio & Seed/Ovule ratio) Germplasm Banks (ex situ Conservation)

(seeds germination & survival of seedlings)

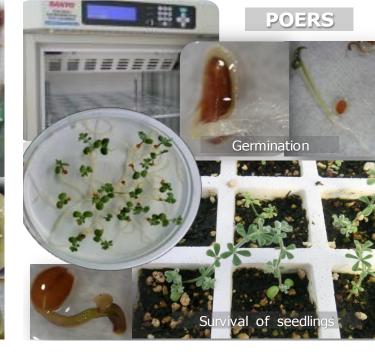
The **THIRD PARTIAL OBJECTIVE** is aimed at:

- EVALUATION OF FEMALE REPRODUCTIVE SUCCESS (SEED SET)
- Pre-emergent (PERS): evaluation of flowers which form fruits and ovules that form seeds:
- Fruit/Flower x Seed/Ovule • Post-emergent (POERS): evaluated by seeds germination

and survival of seedlings

The types of individuals according to sexual or self-incompatibility systems will be compared (hermaphrodite vs female, cob vs pap, pin vs thrum).





THE SEED BANKS (EX SITU CONSERVATION)



Seeds collected in MACFLOR will be stored the Seeds Banks of the participating partners (JBCVC and JBF)

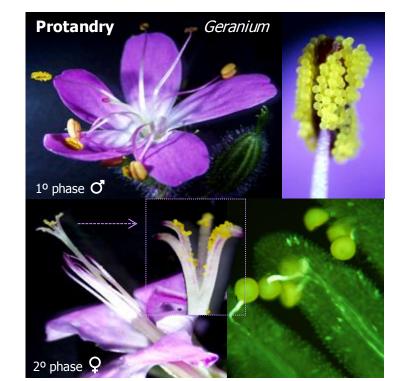


The **SECOND PARTIAL OBJECTIVE** are focused:

FLORAL BIOLOGY & PHENOLOGY. FLORAL VISITORS AND DICHOGAMY

Floral phenology studies require observations in the field (or nursery) and in the laboratory, using specific techniques. Morphological and chromatic changes that occur in the floral whorls (calyx, corolla, androecium and gynoecium) are detected and its physiological meaning is interpreted.

Dichogamy (protrandy or protogyny) is analysed in hermaphrodite and monoecious individuals, as a mechanism that potentially prevents interferences between the androecium and gynoecium and promotes the xenogamy.





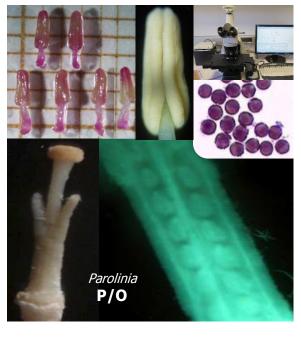
The evaluation of changes in floral whorls, resources of the androecium and gynoecium allow to identify the syndromes and reveal the possible pollination vectors (anemophily, entomophily and/or ornithophily).

* Breeding systems. Self-incompatibility

The breeding systems (autogamy-xenogamy) will be evaluated by Pollen/Ovule ratio (P/O) and, in some species, also by experimental **pollination** with detection of self-incompatibility.

> Experimental cross- and self-pollination allow direct evaluation of xenogamy by SELF-

INCOMPATIBILITY detection. This activity



Index of xenogamy and P/O ratio per flower can be estimated evaluating the number of pollen grains produced by the anthers of a flower in relation to the number of ovules of that same flower or of another flower of the same plant.



requires bagging both the pollen-receiving

It is possible to confirm the nature of the self-incompatibility observing (MO fluorescent staining) the interruption of the **pollen tubes** in the gynoecium of self-pollinated flowers.



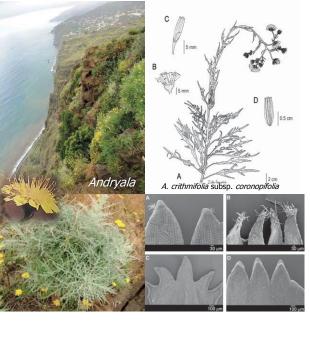
NEOCHAMAELEA. PATERNITY ANALYSIS.

In Neochamaelea, with a complex sexual system, where four sexual phenotypes are present (duodichogamous-DD, protandrous-PA, protogynous-PG and male-M individuals), genetic paternity analysis (microsatellites) will be carried out to check the pollen donors and crossings between sexual phenotypes.

* ANDRYALA. GENETIC DIVERSITY OF GENUS IN **M**ADEIRA

The study of the population genetic diversity in Andryala from Madeira using molecular markers allow to characterize the variability and genetic structure of the populations in Madeira, to test the hybridization betweer

endemic species.





ATLAS & RECOVERY STRATEGIES

The detection of erosive processes in the **REPRODUCTIVE CYCLE** (flowering and fructification) associated with the loss of reproductive success or failures in the formation of viable seeds, closely related with the morphological and functional traits of flowers, will allow to design **RECOVERY STRATEGIES** in the macaronesian endemic species studied in MACFLOR.

These **Recovery Strategies** provides concrete and effective solutions (possibility of low cost) since it would be enough to reinforce the population with those phenotypes or suitable individuals that can "correct" the lack of reproductive success, not only in the short term, but also in the medium and long term, guaranteeing the survival of populations and species





