

Establishment of a Genetic Reserve Network for Sugar Beet Crop Wild Relatives



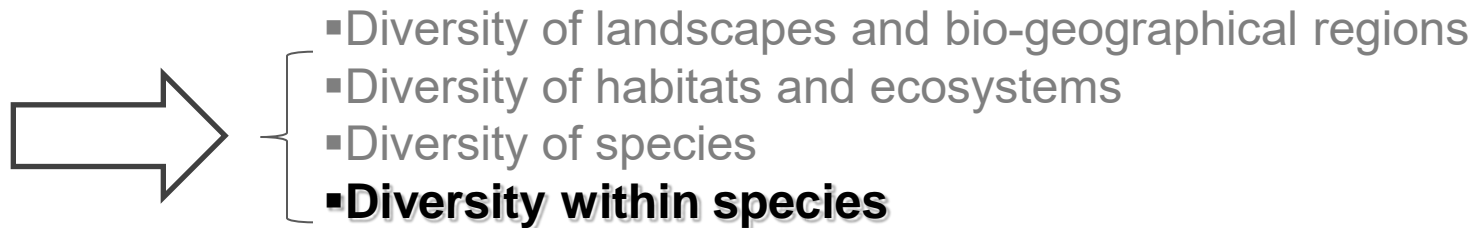
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Definitions

"In situ conservation" means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings [...] where they have developed their distinctive properties (CBD, 1993)



Genetic reserve conservation technique

“The location, management and monitoring of genetic diversity in natural populations within defined areas designated for long-term active conservation”.

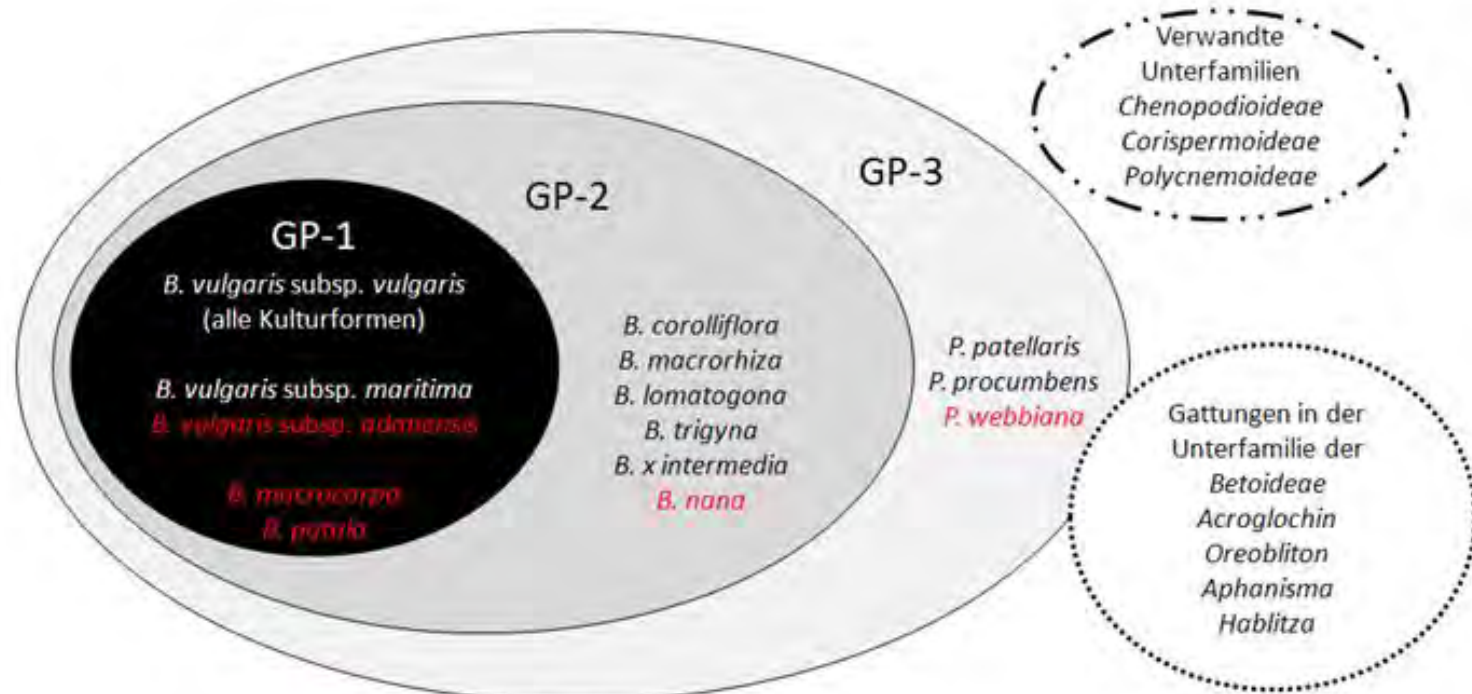
Combines the best elements of the ex situ and in situ conservation strategy.

(Maxted et al., 1997).

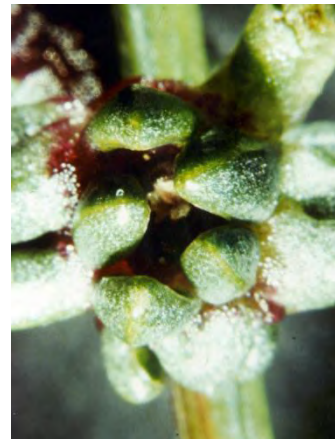
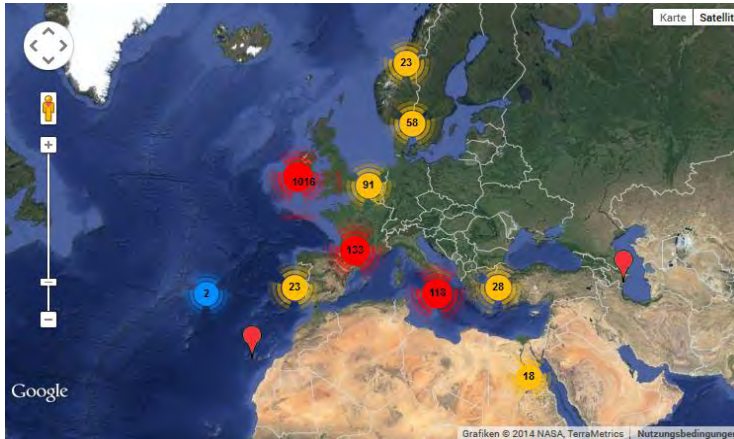


Justification

- Genebanks are technically and politically vulnerable
- CBD and IT
- Need to combine *ex situ* (static, user-friendly) and *in situ* (dynamic, evolution) conservation for a group of target species (gene pool).
- Compensation of risks
- Adaptation of wild species generates novel genetic variation useful to breeding

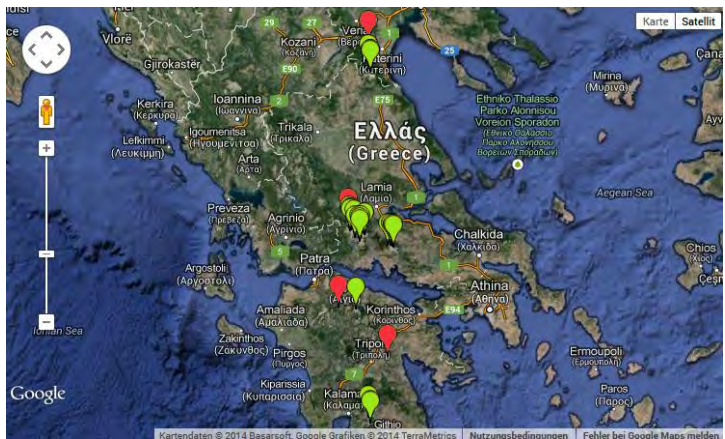
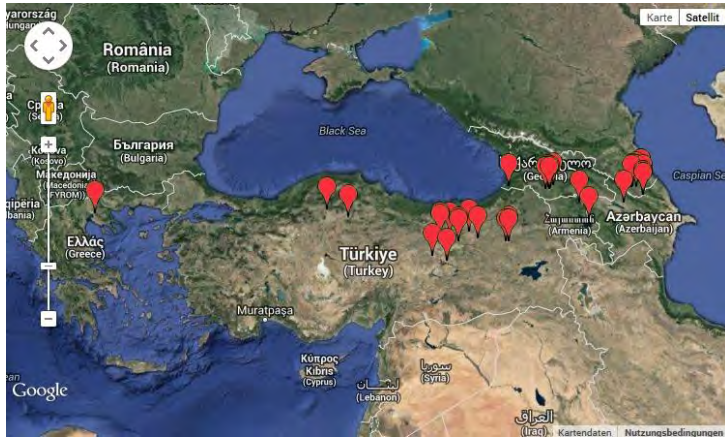


Distribution and habitats



Examples for crop wild relatives (CWR) of genepool 1 (GP-1).
Upper part: Distribution of the sea beetle. Habitat at the Baltic Sea coast.
Lower part: Distribution of *B. macrocarpa*. Habitat in Portugal.

Distribution and habitats



Examples for genepool 2 (GP-2).
Upper part: Flowering *B. macrorhiza*, Habitat in Daghestan.
Lower part: *B. nana*. Habitat in Greece, Chelmos Mountain .

Distribution and habitats



Examples for genepool 3 (GP-3).

Upper part: Seed of *Patellifolia patellaris*. Habitat in Tenerife.

Lower part: *P. procumbens*. Habitat in Tenerife, Roque de Bodegas.

Objectives

Identification of a set of populations representing the genetic diversity of a species

Most **A**ppropriate crop **W**ild relative **P**opulations (MAWP) (Maxted et al., 2015)

Application of the gene pool approach (Kell et al., 2012)

Step 1: taxon delineation

Step 2: selection of target taxa

Step 3: diversity analysis

Step 4: selection of target sites

Identification of MAWP. Step 2 - selection of target taxa.

| Taxon | IUCN threat category (Bilz et al., 2011) | GP |
|---------------------------|---------------------------------------------|----|
| B. vulgaris ssp. vulgaris | LC | 1 |
| culton group LB | | 1 |
| culton group GB | | 1 |
| culton group FB | | 1 |
| culton group SB | | 1 |
| B.v. ssp.maritima | LC | 1 |
| B. adanensis | VU | 1 |
| B. macrocarpa | EN | 1 |
| B. patula | CR | 1 |

Identification of MAWP. Step 2 - selection of target taxa.

| Taxon | IUCN threat category (Bilz et al., 2011) | GP |
|-----------------|---------------------------------------------|----|
| B. corolliflora | | 2 |
| B. macrorhiza | | 2 |
| B. lomatogona | | 2 |
| B. trigyna | DD | 2 |
| B. intermedia | | 2 |
| B. nana | VU | 2 |
| | | |
| P. procumbens | LC | 3 |
| P. webbiana | CR | 3 |
| P. patellaris | LC | 3 |

Identification of MAWP. Step 2 - selection of target taxa.

Prioritization of taxa using only the criteria “threat status” and “absence of crossing barriers between the cultivated and wild species” would generate a short list:

| Taxon | IUCN threat category (Bilz et al., 2011) | GP |
|---------------|---------------------------------------------|----|
| B. adanensis | VU | 1 |
| B. macrocarpa | EN | 1 |
| B. patula | CR | 1 |

Identification of MAWP. Step 2 - selection of target taxa.

Plant genetic resources conservation aims at the conservation of genetic variation required to improve crop varieties.

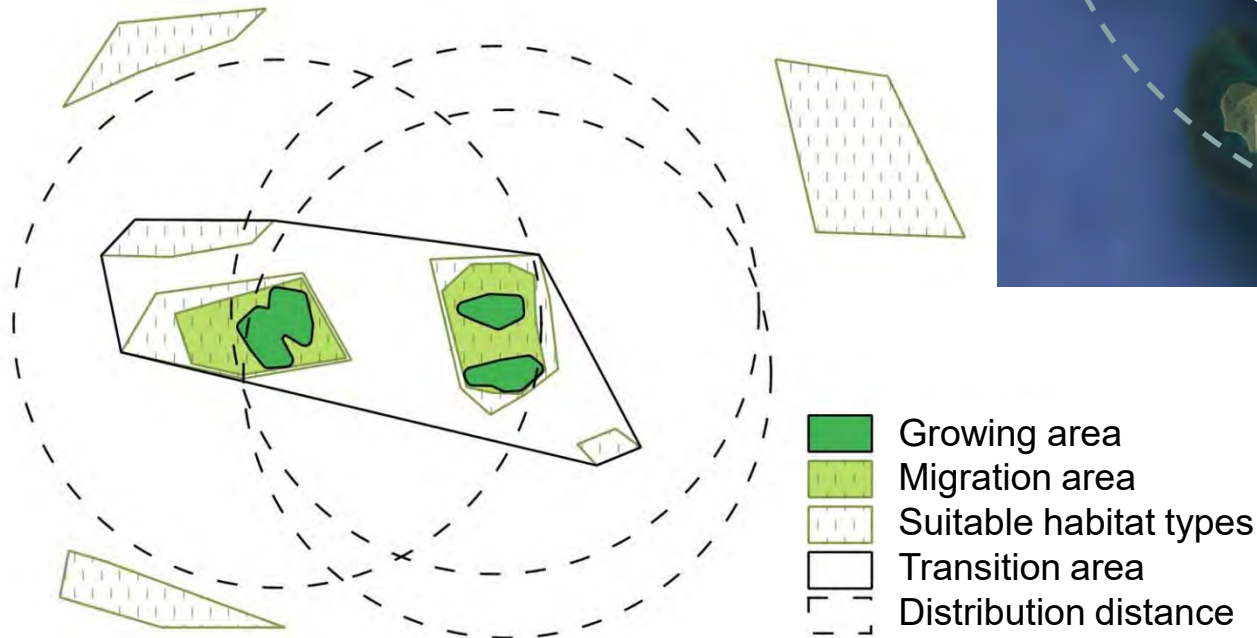
The ultimate goal of plant genetic resources conservation programs is to conserve a representative sample of intraspecific diversity of all CWR belonging to the gene pool of cultivated beets.

To this end we need

- (i) to quantify the degree of uniqueness of the populations with respect to their genetic composition,
- (ii) to locate and delineate genetic reserves and
- (iii) to organize a network of genetic reserve sites to achieve the complementarity between *ex* and *in situ* conservation

Identification of MAWP. Step 2 - selection of target taxa.

Model of a genetic reserve (modified after Maxted et al. (1997) (below) and a recommended genetic reserve site located at the Kalundborg Fjord in Denmark.



Identification of MAWP. Step 2 - selection of target taxa.

| Taxon | Chromosome number | Diversity studies and marker types used (incomplete) |
|-----------------------------------------|-------------------|------------------------------------------------------|
| <i>B. vulgaris</i> ssp. <i>vulgaris</i> | 18 | |
| culton group LB | 18 | Few (RFLP, SSR, mitochondrial minisatellites) |
| culton group GB | 18 | Few (RFLP, SSR, mitochondrial minisatellites) |
| culton group FB | 18, 27 | Some (RFLP, SSR, DArT?) |
| culton group SB | 18, 27 | Some (RFLP, SSR) |
| <i>B.v.</i> ssp. <i>maritima</i> | 18 | Many (isozymes, RFLP, SSR, DArT) |
| <i>B. adanensis</i> | 18 | Few (isozymes, RFLP, SSR, DArT) |
| <i>B. macrocarpa</i> | 18, 36 | Few (isozymes, RFLP, SSR, DArT) |
| <i>B. patula</i> | 18 | Few (isozymes, RFLP, SSR) |

Identification of MAWP. Step 2 - selection of target taxa.

Step 3: diversity analysis

| Taxon | Chromosome number | Diversity studies and marker types used (incomplete) |
|------------------------|-------------------|------------------------------------------------------|
| <i>B. corolliflora</i> | 36 | Few (isozymes, RFLP) |
| <i>B. macrorhiza</i> | 18 | Few (isozymes, RFLP) |
| <i>B. lomatogona</i> | 18 | Few (isozymes, RFLP) |
| <i>B. trigyna</i> | 45, 54 | Few (isozymes) |
| <i>B. intermedia</i> | 36, 45 | Few (isozymes) |
| <i>B. nana</i> | 18 | Few (SSR, RFLP) |
| | | |
| <i>P. procumbens</i> | 18 | Few (SSR, RFLP) |
| <i>P. webbiana</i> | 18 | Few (SSR, RFLP) |
| <i>P. patellaris</i> | 36 (18, 27) | Few (SSR, RFLP) |

Identification of MAWP. Step 2 - selection of target taxa.

Quantity of genetic marker data and information

- Beta section Beta: high
- Beta section Corollinae: low
- Patellifolia: low (compared to Beta section Beta)

Identification of MAWP – a decision problem!

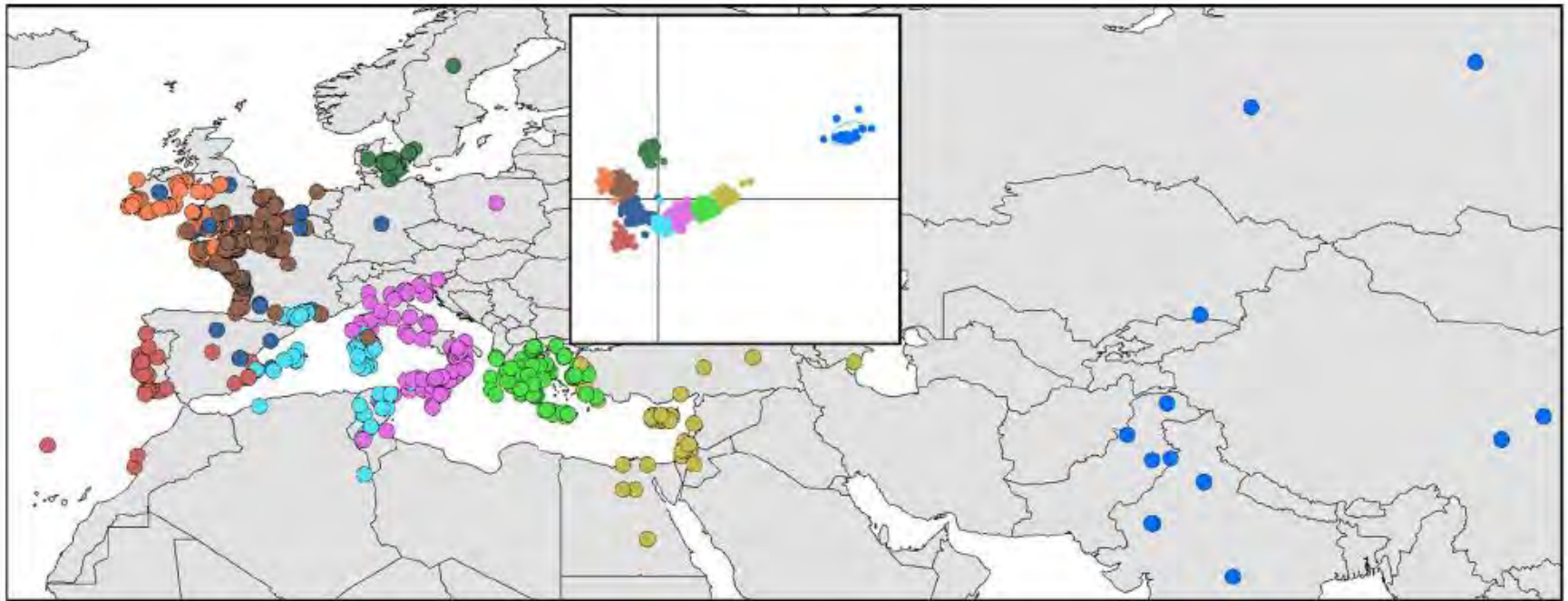


Fig. 4 Genetic structure of the 923 well-classified accessions of *B. vulgaris* subsp. *maritima* estimated with a discriminant analysis of principal components (DAPC) using $K = 10$ clusters as estimated by the K -means methods. The *inset* shows the first and second discriminant functions

Andrello et al. (2015), DArT, clinal variation from S to N and W to E

Recommend few accessions for each of the groups?

Identification of MAWP – a decision problem!

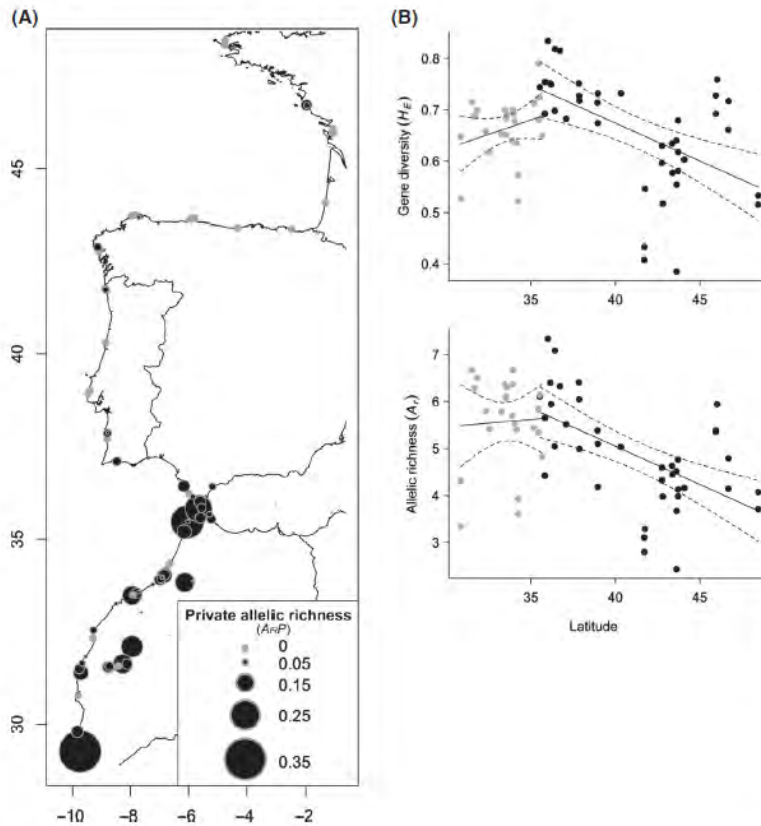


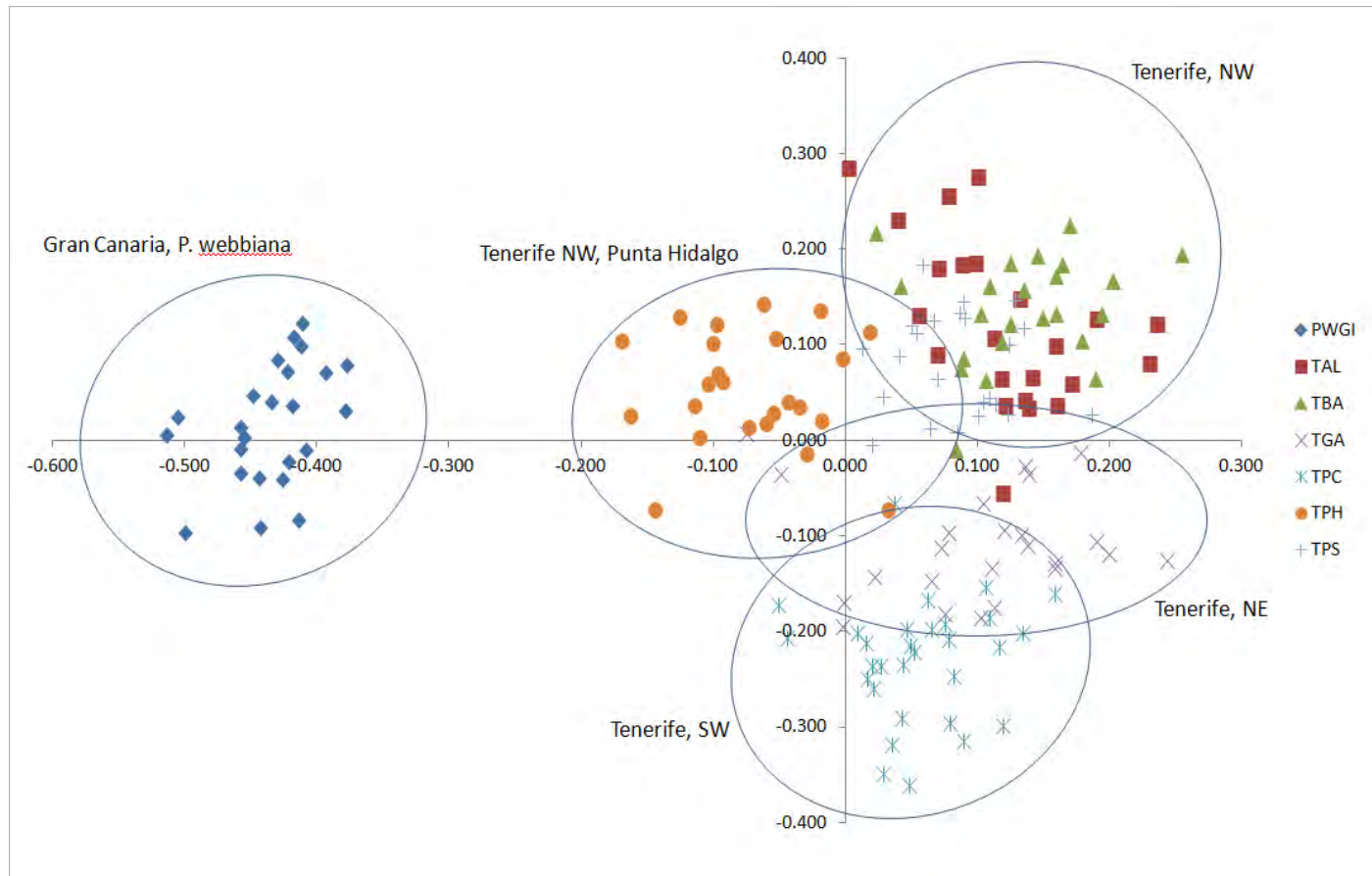
Figure 3. Spatial patterns of genetic diversity. (A) Nuclear private allelic richness (A_{rP}) of *B. vulgaris* subsp. *maritima* populations is proportional to the size of the dot mapped onto the population location. (B) Linear regressions of gene diversity (H_E) and allelic richness (A_r) based on nuclear polymorphism with respect to latitude. Black dots represent genetic diversity values for northern populations, from Brittany to northern Moroccan populations at the Strait of Gibraltar (populations labelled 1–40 in Fig. 1). Gray dots represent values for Moroccan populations at lower latitudes (populations labelled 43 to *t*). Dashed lines indicate the 95% confidence intervals.

Leys et al. (2014), SSR, more rare alleles in Moroccan populations

Recommend populations with high number of private alleles?

Identification of MAWP – a decision problem!

P. procumbens/P. webbiana, factorial analysis



Identification of MAWP – a decision problem!

Genetic distance measure Δ (Gregorius et al., 2003)

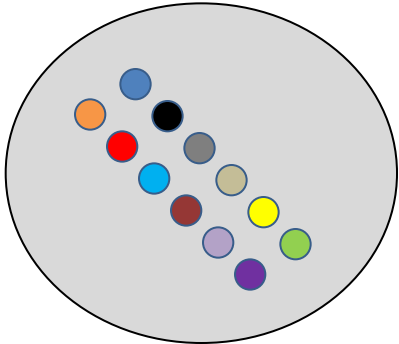
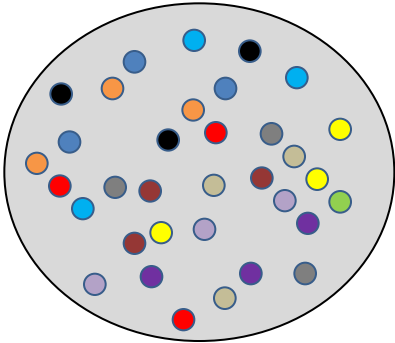
For a specific trait and a pair of populations, the pairwise genetic distance (Δ) equals the minimal extent to which the genetic types (e.g., alleles of a gene) of individuals in one population must be altered to obtain the composition of genetic types in the other (Gillet and Gregorius 2008).

Computer program DifferInt (Gillet, 2013a, b)

Identification of MAWP – a decision problem!

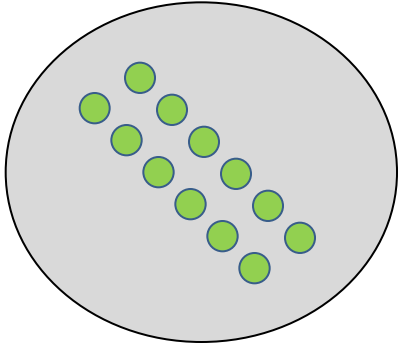
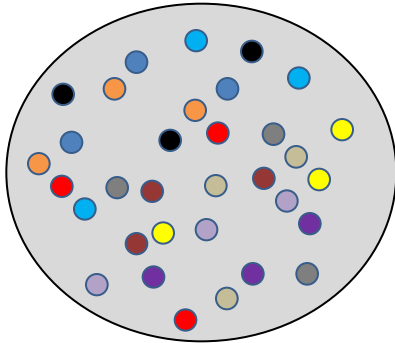
Illustration of Δ_j

The complement: all populations except for population A



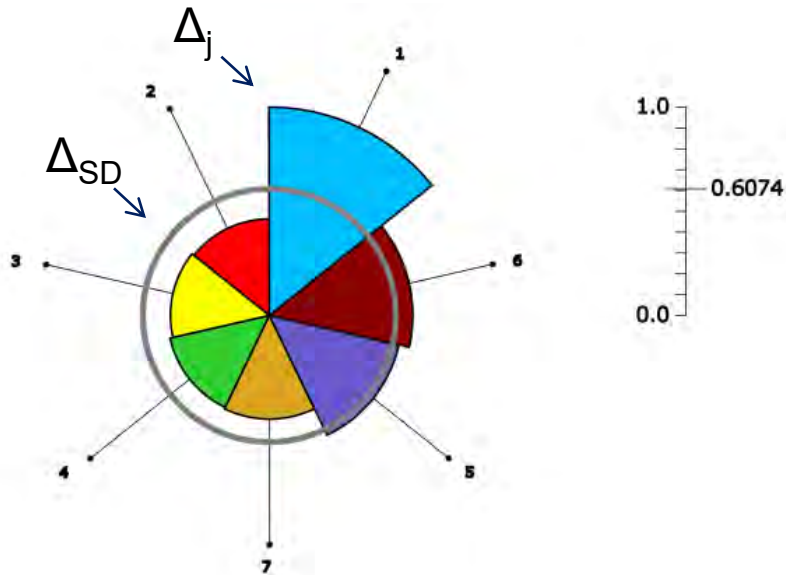
$$\Delta_A = 0$$

The complement: all populations except for population B



$$\Delta_B = 1$$

Identification of MAWP – a decision problem!



Complementary
compositional
differentiation

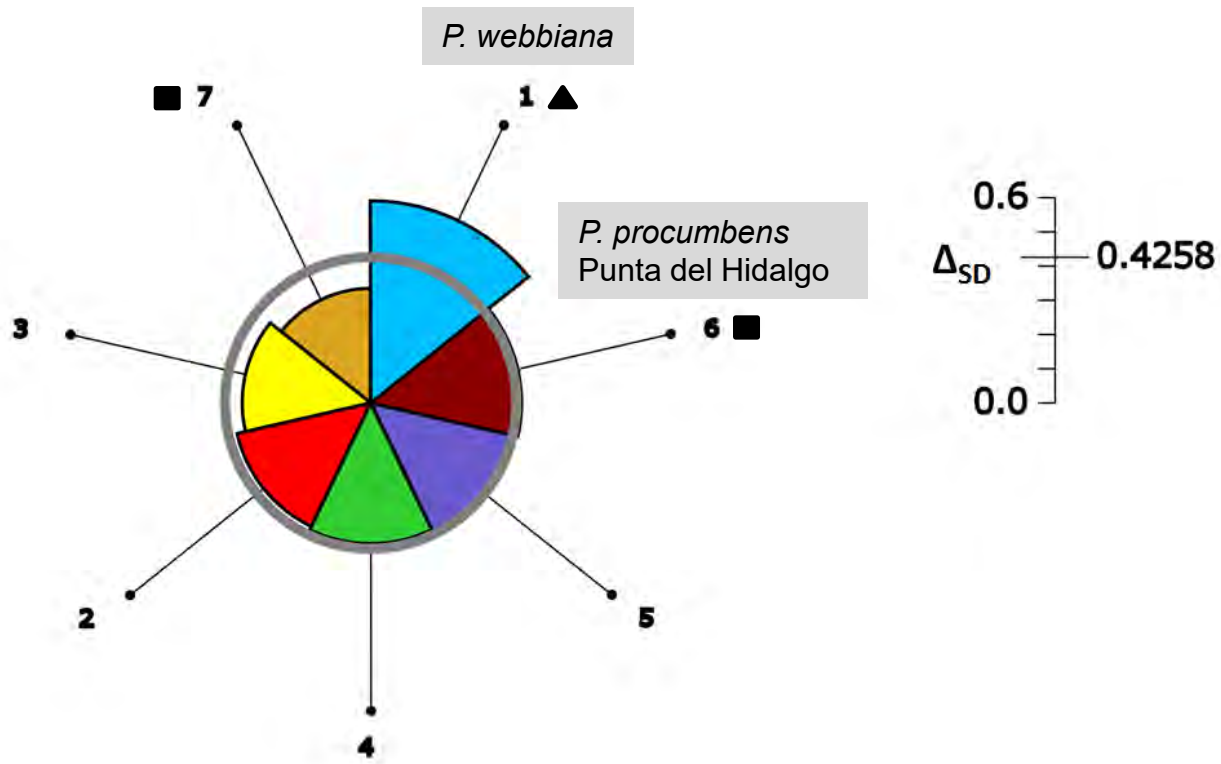
$\Delta_j = 0$: The genetic composition of the occurrence j does not differ from the average of the remaining pooled occurrences, the complement.

$\Delta_j = 1$: The genetic composition of the occurrence j differs completely from its complement.

Δ_{SD} : mean of all radii

Identification of MAWP – a decision problem!

Differentiation in *P. procumbens/webbiana*, 22 SSR



Recommend populations with based on the representativity / uniqueness of their genetic information?

Identification of MAWP – a decision problem?

Table 1. Sites proposed for the establishment of genetic reserves for the focal species of the four target taxa. Type of area specifies the degree of protection: Natura 2000 refers to sites of community interest (SIC) under Directive 92/43 belonging to Natura 2000 network; CDDA (Common Database on Designated Areas) refers to protected areas under national legislation, and “not protected” to locations outside protected areas.

| Sitename | Type | Sitecode | Country | Focal species |
|---------------------------------------------------------------------------|---------------|-----------|----------|--------------------------------------------------------------------------|
| West of Lake Comunelli | not protected | - | Italy | <i>Avena insularis</i> Ladiz. |
| Mountain Giase | | | | |
| Private land not protected | | | | |
| Los Alcornocales | | | | |
| Estrecho | | | | |
| Alykos Potamos - Agios Sozomenos | | | | |
| Alykes Larnakas | Natura2000 | CY6000002 | Cyprus | <i>Avena ventricosa</i> Coss. |
| Athalassa National Forest Park | CDDA | CYP14837 | Cyprus | <i>Avena ventricosa</i> Coss. |
| Doñana | Natura2000 | ES0000024 | Spain | <i>Avena longiglumis</i> Durieu. <i>Avena hirtula</i> Lag. |
| La Breña y Marismas del Barbate | Natura2000 | ES6120008 | Spain | <i>Avena longiglumis</i> Durieu. <i>Avena hirtula</i> Lag. |
| Ría Formosa - Castro Marim | Natura2000 | PTCON0013 | Portugal | <i>Beta macrocarpa</i> Guss. |
| Salinas de Santa Pola | Natura2000 | ES0000120 | Spain | <i>Beta macrocarpa</i> Guss. |
| Cabo de Gata - Níjar | Natura2000 | ES0000046 | Spain | <i>Beta macrocarpa</i> Guss. |
| Archipiélago Chinijo | Natura2000 | ES7010045 | Spain | <i>Beta macrocarpa</i> Guss. |
| Costa del Norte de Fuerteventura | Natura2000 | ES0000348 | Spain | <i>Beta macrocarpa</i> Guss. |
| Amagro | Natura2000 | ES7010011 | Spain | <i>Beta macrocarpa</i> Guss. |
| Lomo del Carretón | Natura2000 | ES7020037 | Spain | <i>Beta macrocarpa</i> Guss. |
| Interían | Natura2000 | ES7020081 | Spain | <i>Beta macrocarpa</i> Guss. <i>Beta macrocarpa</i> Guss. |
| Anaga | Natura2000 | ES0000109 | Spain | <i>Beta procumbens</i> (Hornem.) A.J.Scott, Ford-Lloyd & J.T.Williams |
| Notoanatolikos Parnassos - Ethnikos Drymos Parnassou - Dasos Tithoreas | Natura2000 | GR2450005 | Greece | <i>Beta nana</i> Boiss. & Heldr. |

<http://www.agrobiodiversidad.org/aegro/>

28 genetic reserves for *Beta* / *Patellifolia* recommended

Iriondo et al. (2011), annex 16 to the final AEGRO project report

Recommendations based on assumed adaptedness and expert knowledge

Identification of MAWP. Step 4: selection of target sites

The final choice of a sites depends on

- (i) the capacity of nature conservation agencies and/or
- (ii) the willingness of land owners to support the establishment and long-term operation of a genetic reserve.

(i) and (ii) can outweigh genetic arguments.

The establishment of genetic reserves at the selected target sites must follow the guidelines for participatory nature conservation project planning.

Step 5: establishment of genetic reserves



Genetische
Erhaltungsgebiete
Wildsellerie

Field of Cooperation

Potential cooperation partners

Nature conservation agencies, organisations, associations, land owners, land users, neighbours

Involved parties

Project initiator, projekt funding agency, project partners

Supporters

Advisory boards: policy, organisations, association; media

Contracting body

Such as Ministries

Basis

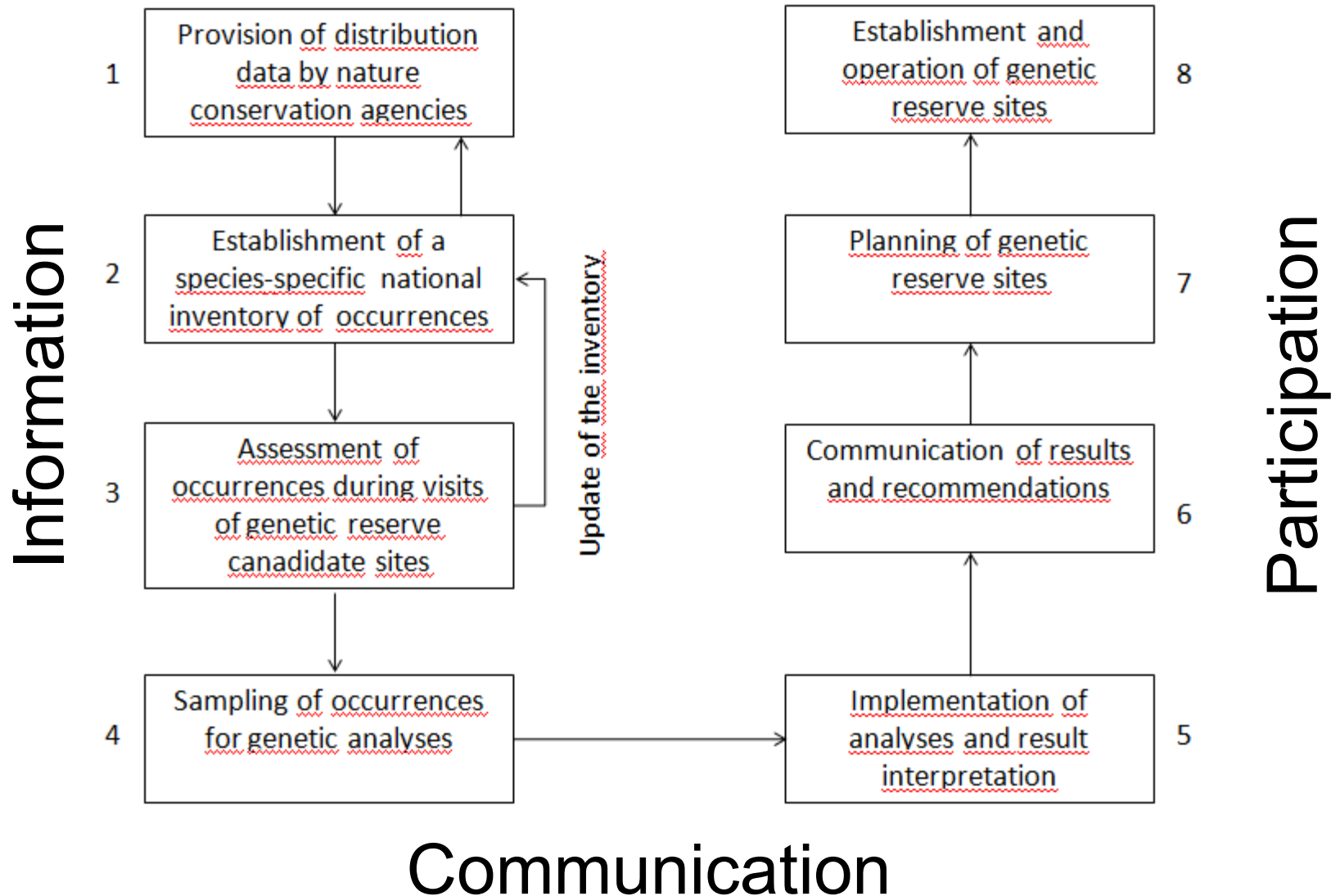
Derived R&D programs
CBD (1992) and the International Treaty (2004)



Genetische
Erhaltungsgebiete
Vielfalt bewahren



Step 5: establishment of genetic reserves



Conclusions

The choice of sites, the establishment and operation of genetic reserves is within the responsibility of each European country.

The function of the **ECPGR working group on *Beta*** would be to improve the knowledge basis by coordinating joint activities such as the

- (i) establishment of up-to-date national (species-specific) CWR inventories,
- (ii) assessment of the conservation status in the country,
- (iii) investigation of the spatial patterns of genetic differentiation, and the
- (iv) development of genetic decision criteria required to identify MAWP.



Thank you for your attention