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PLANTS FOR THE FUTURE – A FUTURE FOR OUR PLANET

TOWARDS A PROTOCOL FOR GENETIC MANAGEMENT OF *EX SITU* LIVING PLANT COLLECTIONS

There is an urgent need for worldwide assessment of genetic diversity of *ex situ* living plant collections and comparison with wild populations, especially for threatened species. The Botanical Garden of Ghent University, Belgium is addressing this need.

Esteban Martínez climbing a tree to collect a newly discovered Hydrangea *species on the volcano Tacaná in Chiapas state in southeast Mexico near the border with Guatemala. Stems with leaves of this species are visible on all the trunks (Marie-Stéphanie Samain).*

Introduction

It is estimated that one third of all plant species are threatened with extinction and yet plants continue to be underrepresented in conservation debates and policies. However, global initiatives, such as the Global Strategy for Plant Conservation (GSPC) are aiming to bring a significant change. As stated in Target 8 of the GSPC, at least 75% of threatened plant species should be present in *ex situ* collections. Botanical gardens play a key role in conservation of plant diversity, but only about 30% of globally threatened plant species are currently held in their living collections (Oldfield, 2010). To be of conservation value, living collections must be genetically representative. Although information on genetic diversity in *ex situ* collections is scarce, it is thought that diversity is low in collections of numerous species. Moreover, besides the many studies and initiatives for specific taxa, our knowledge about the genetic diversity of threatened plant species in general is quite limited. This lack of insight into genetic diversity of threatened wild plant species contrasts with the broad knowledge about crops and their wild relatives. This fact is also reflected by GSPC Target 9, which specifically states that 70% of the genetic diversity of crops, including their wild relatives and other socio-economically valuable plant species, should be conserved. We still have a long way to go to understanding genetic diversity in threatened plants, conserving an important part of this diversity in *ex situ* collections, defining which percentage of genetic diversity might be feasible and/or desirable to aim for, and finally making this available for conservation and restoration projects.

A happy team of botanists and local children after discovering a new Hydrangea *species on the volcano Tacaná in Chiapas state in southeast Mexico near the border with Guatemala (Paco Najarro).*

Setting the stage

The GSPC 2011-2020 states that:

> *"Without plants there is no life. The functioning of the planet, and our survival, depends upon plants."*

Achieving Target 8 of the GSPC depends on the existence of genetically representative collections, and these are essential for recovery and restoration work. Our focus should therefore be on assessing and ensuring the conservation value of *ex situ* collections (Sharrock *et al*., 2010).

Assessments of a representative sample of plant species will provide a basis for an initial estimation of a baseline, and to monitor progress towards this target. Therefore, toolkits under this target need to include protocols for genetic management of *ex situ* collections and consequent reintroductions.

One of the suggested milestones to serve as a step towards the 2020 target is that *ex situ* collections of all critically endangered species should be genetically representative of the species (SBSTTA, 2010). However, preliminary observations and data suggest that this currently is not the case in many collections, as the genetic diversity from wild population to *ex situ* collections 'gradually' decreases (= genetic bottleneck) as a consequence of 1) habitat destruction narrowing genetic diversity and subsequent evolution (= genetic erosion), 2) collection bias ('easy' localities, selection of morphological variation which is not necessarily reflected by genetic variation), 3) cultivation bottleneck (accessions dying because of unsuitable conditions). Hence, the establishment of a protocol guiding genetic management during the different steps (wild population – *ex situ* collection – reintroduction) is essential for implementation of Target 8 of the GSPC and, ultimately, for safeguarding the future of our planet.

Towards an assessment for flagship groups: *Hydrangea, Magnolia* and Cactaceae

The Botanical Garden and the Research Group Spermatophytes of Ghent University (Belgium) are increasingly involved in conservation of the plant groups which are housed in their living collections and studied in ongoing projects, such as *Peperomia* (Samain *et al*., 2010), *Hydrangea* (Red List project in cooperation with BGCI, including a specific conservation project in Mexico), and *Aristolochia* (Rivera Hernández & Samain, 2011). Our strategy, to combine fundamental botanical research with conservational efforts, has also led to the establishment of a project, initiated in 2011, which will be continued and accelerated thanks to the support of the Fondation Franklinia. Within this project we aim to compare genetic diversity between wild populations and *ex situ* collections of three unrelated Angiosperm groups with different life histories and growth forms. A range of genomic tools will be applied to wild and *ex situ* individuals of selected species of the flagship groups *Hydrangea, Magnolia* and Cactaceae to enable rapid characterization of genetic variation, providing 1) support for specific conservation actions, and 2) general guidelines and a protocol on genetic management for acquiring accessions for *ex situ* collections, in order to be used by collectors and botanical gardens worldwide in the framework of the achievement of Target 8 of the GSPC.

Sampling and lab work

The three plant groups being studied are considered as priority groups for BGCI, the IUCN/SSC Global Trees Specialist Group and the International Organization for Succulent Plant Study (IOS), with whom we cooperate. These groups are rather well-studied taxonomically and are also very important in the horticultural trade, with especially Cactaceae also suffering from illegal harvest and trade. Despite the fact that these groups are a priority for conservation, the genetic diversity within *ex situ* collections is almost entirely unknown, and has not been compared with wild populations. There is clearly an urgent need for this research.

Collecting Hydrangea aspera *with local botany students on mountain Taiping Shan, Taiwan (Eduardo Cires).*

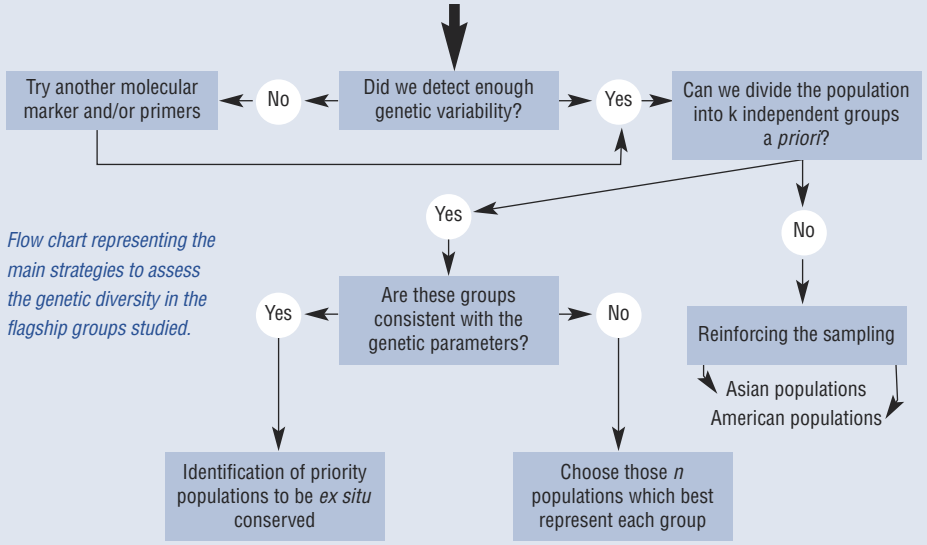
Characteristic	RFLP	Microsatellite	AFLP	ISSR	PCR sequencing
Genomic abundance	High	Medium	Very high	Medium	Very high
Part of genome surveyed	Low copy coding regions	Whole genome	Whole genome	Whole genome	Whole genome
Amount of DNA required	High	Low	Medium	Low	Low
Type of polymorphism	Single base changes, insertion, deletion	Changes in length of repeats	Single base changes, insertion, deletion	Single base changes, insertion, deletion	Single base changes, insertion, deletion
Level of polymorphism	Medium	High	Very high	High	Very high
Inheritance	Codominant	Codominant	Dominant	Dominant	–
Ease of use	Labour intensive	Easy	Difficult initially	Easy	Easy
Automation possible	Low	High	Medium	Medium	High
Cost of automation	High	High	High	Medium	High
Development costs	Medium	High	Low	Low	High
Reproducibility (reliability)	High	High	High	Medium to high	High
Level of training required	Low	Low/Medium	Medium	Low	High
Cloning and/or sequencing	Yes	Yes	No	No	Yes
Radioactive detection	Yes/No	Yes/No	Yes/No	No	Yes/No
Proprietary rights status	No	No (some licensed)	Licensed	Licensed	No (some licensed)

Table 1. Comparative assessment of some of the salient characteristics of different molecular genetic screening techniques: RFLP (or Restriction fragment length polymorphism), Microsatellites or simple sequence repeats (SSRs), AFLP (or Amplified fragment length polymorphism), ISSR (or Inter Simple Sequence Repeat) and PCR sequencing.

> *"Around one-third of the globally threatened species that are found in botanic garden collections are recorded in only one collection."*

Global and/or local Red Lists are available for each group (*Magnolia*: Cicuzza *et al*., 2007; Cactaceae: e.g. IUCN, 2011) or are being prepared (*Hydrangea s.l.*). These groups represent different unrelated families of Angiosperms, with different habitat preferences and a range of life history traits. Within each group, taxa are selected based on: 1) presence in a representative number of public and accessible private *ex situ* collections (botanical gardens, arboreta, etc.), 2) well-documented origin of the accessions, and 3) availability of material verified by specialists. Because of the second criterion, the quantity of useful accessions available for the research is much reduced, but we believe it is not useful to include samples without documented origin, as their value, for example in reintroduction projects, is highly doubtful. Additionally, *Magnolia* and *Hydrangea* individuals are plants with life spans of several hundred years, predating fragmentation events, so we will obtain reliable genetic information that may help genetic restoration projects (e.g. López *et al*., 2008).

Availability in botanical garden collections has been checked via the PlantSearch module on the BGCI website (http://www.bgci.org/plant_search.php) or via direct and ongoing communication with gardens. A final selection criterion is that most of the species are 'Vulnerable', 'Endangered' or 'Critically Endangered' according to IUCN Red List categories and criteria, hence they are priority species for conservation and for use in recovery and restoration work. Furthermore, the genetic diversity of these rare species will be compared with some more common or widespread species.



Flow chart representing the main strategies to assess the genetic diversity in the flagship groups studied.

Field work has already been or will be performed in countries and areas where the Research Group has extensive field experience and local cooperation partners (Latin America and East Asia). Within the wild populations, a representative sample of individuals will be collected, meanwhile in the *ex situ* collections we aim to include an equal number of accessions from as many gardens as possible. In addition, our work on *Hydrangea* will also help to build new living collections, e.g. in Mexico, supported by the Mohamed Bin Zayed Species Conservation Fund.
Molecular tools provide valuable data on diversity through their ability to detect variation at the DNA level. Identification is of fundamental importance in diversity studies in a variety of different ways. For evaluation of species diversity, it is essential that individuals can be classified accurately. The identification of taxonomic units and endangered species, whose genetic constitution is distinct from their more abundant relatives, is important in the development of appropriate conservation strategies. Taking advantage of current molecular techniques, relatively quick assessments of genetic diversity in plants can be performed using well-sampled material and ready-made protocols, as long as the most limiting factor, funding, is available. For each flagship species, different molecular techniques (e.g. RFLP, microsatellites, AFLP, ISSR, PCR sequencing) are currently being tested and/or developed, with comparisons of cost versus data yielded, ease of use, and applicability for conservation

research (Table 1). For the elaboration of these techniques, either in-house experience has been developed or collaboration with experienced research groups has been set up.

Outlook – Connecting the dots

To our knowledge, this is the first comprehensive study of this kind, with a constant evaluation of the different techniques, in order to assess the progress of the project (see Figure 1). The expected results can be subdivided in two levels: i) specific results for each group: they will provide insight into genetic diversity and history, providing a basis for further conservation work; ii) comparison of different unrelated groups with different life histories and growth forms: this approach will lead to a general overview of the genetic diversity available in wild populations and *ex situ* collections, as well as of the specific problems involved with decreasing genetic diversity from the wild to the collection, information which will be extremely useful for implementation of Target 8 of the GSPC.

Some important specific outcomes are listed here: 1) assessment of percentage of genetic diversity present in *ex situ* collections worldwide for the three plant groups under study, 2) identification of priority populations for *ex situ* conservation, 3) definition of a genetically representative collection (compared to Target 9 of the GSPC stating that 70% of the genetic diversity of crops and other socio-economical valuable plant species should be conserved), 4) indication of the number of samples that needs to be collected in the wild to obtain a genetically representative collection, 5) testing the hypothesis that endangered species with narrow distribution are genetically limited, 6) conservation of flagship species, and finally, 7) reports and publications in cooperation with e.g. BGCI, the IUCN/SSC Global Trees Specialist Group and the IOS.
Last but not least, it has already become clear during this pilot year 2011 that this study will lead to further international cooperation with many interested gardens and researchers.

> *"We expect to form a scientific network studying genetic diversity in plants for conservation purposes under the auspices of BGCI."*

Please feel free to contact us if you would like to cooperate, exchange experiences, or if you have comments or questions.

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Mountain Wawu Shan, Sichuan, China, location of several Hydrangea *specimens (Eduardo Cires).*

Marie-Stéphanie Samain preparing herbarium specimens of a new Hydrangea *species of Los Tuxtlas, state of Veracruz, Mexico (Esteban Martínez).*

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