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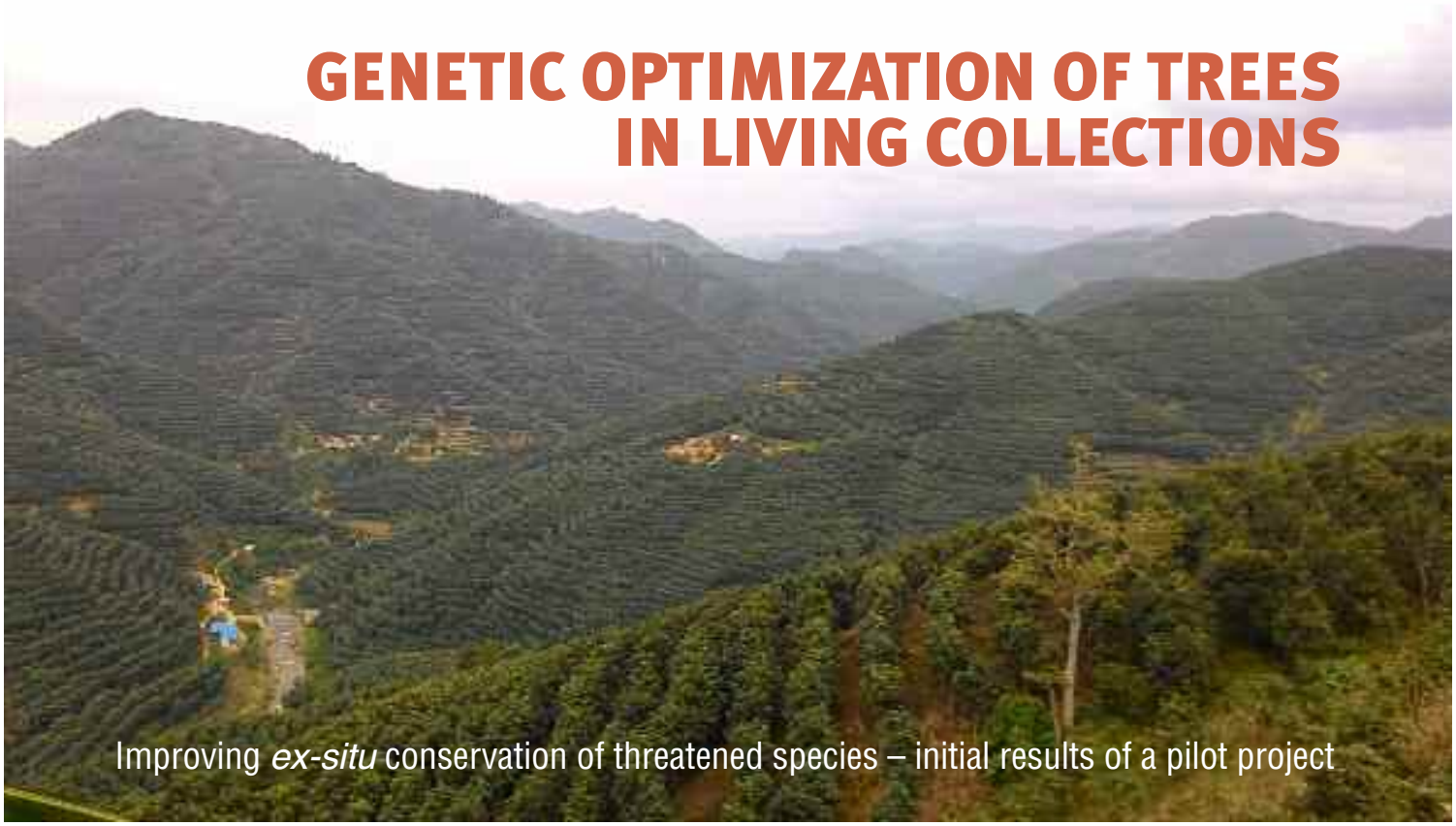
GENETIC OPTIMIZATION OF TREES IN LIVING COLLECTIONS



Improving *ex-situ* conservation of threatened species – initial results of a pilot project

The extent of rubber plantation expansion in Xishuangbanna, China

Introduction

In the face of rapid habitat loss and heightened threats to biodiversity, *ex-situ* conservation has become an increasingly important strategy to preserve viable populations of threatened taxa. Ideally, *ex-situ* collections should include genetically diverse individuals, in order to prevent inbreeding (Krauss *et al*., 2002), maintain the long-term evolutionary potential of the species (Enßlin *et al*., 2011) and to ensure the success of future reintroduction efforts (Hogbin & Peakall, 1999). However, the high cost of molecular work and the absence of molecular markers that can be used for all angiosperm species have prevented genetic screening from being a routine procedure in establishing *ex-situ* populations (Heywood & Iriondo, 2003).

The Zero Extinction Project in Xishuangbanna, China

Xishuangbanna is a plant diversity hotspot, supporting 10% of China's angiosperm flora. Agricultural expansion in recent decades had resulted in habitat loss and fragmentation, threatening the survival of native flora. To ensure the long-term survival of these threatened species, the Xishuangbanna Tropical Botanical Garden (XTBG) initiated the "Zero Extinction Project", which aims to prevent extinction in Xishuangbanna (Fig. 1). Central to this project is the *ex situ* conservation of threatened species in the living collection within XTBG, which is mainly intended for species with recalcitrant seeds, i.e. those with drying-sensitive seeds that cannot be stored in the seed bank.

The need for genetic optimization

Conserving the maximum genetic diversity of threatened species in the living collection is a challenging undertaking. The main technical issue stems from the shear diversity and number of the species involved. An assessment of the conservation status of 3,851 native angiosperm taxa in Xishuangbanna identified 106 taxa as

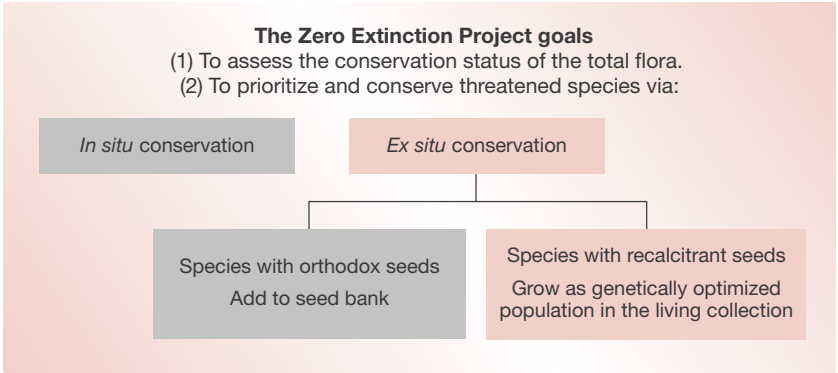


Figure 1: Components of the Zero Extinction Project in Xishuangbanna Tropical Botanical Garden. Genetic optimization aims to improve ex situ *conservation of species with recalcitrant seeds*

endangered or critically endangered and 493 taxa as vulnerable to extinction. Many of these threatened species are large trees and hence space is a major constraint to the number of individuals a botanical garden can preserve. This places great priority on genetically optimizing the living collection, i.e. ensuring that the genetic diversity of a species is represented by the least number of individuals. Fig. 2 outlines a simple work flow for genetic optimization of living collections.

The search for a practical genetic optimization protocol

Traditionally, candidate individuals for *ex-situ* conservation are genotyped with species-specific, fast-mutating microsatellites markers to select for a genetically diverse subgroup among them (Storme *et al*., 2004; Dreisigacker *et al*., 2005). However, with a large number of target species from across the plant phylogeny, employing species-specific markers for genetic screening is too labour-intensive, time-consuming and costly for the process to be practical. Consequently, genetic optimization of the entire living collection remains a model practice that is yet to go beyond textbooks and technical guidelines. Therefore, there is a need for a genotyping protocol that is rapid, universal and cheap.

Aglaia teysmanniana *sapling growing on limestone substrate*

In September 2014, the Center for Integrative Conservation in XTBG started exploring potential genotyping protocols. We focused our search on methods that utilize next generation sequencing (NGS), with the expectation that genotyping costs via NGS will become affordable and the technology accessible to many botanical gardens in the near future. By enabling botanical gardens to increase the capacity of their living collections without a substantial investment in space, genetic optimization represents a large technological step forward in the *ex situ* conservation of tropical plant species. Currently, the Zero Extinction Project initiated by XTBG has been replicated in other gardens in the Chinese Union of Botanical Gardens. Therefore, genetic optimization can potentially be widely applied in these botanical gardens.

The requirement for universality narrowed down our methodological options to genotyping-by-sequencing, either with Restriction-site associated DNA sequencing (RAD-seq) (Baird *et al*. 2008) or a newly developed PCR-based method termed MIG-seq (Suyama and Matsuki in preparation). Both methods generate large numbers of single nucleotide polymorphisms (SNP) loci, can be used without any prior knowledge of genomic sequences, and promise cheap and fast output. Data can be obtained within a month from DNA extraction, and can cost less than 15 USD per sample with multiplexing (Henri *et al*., 2015; Suyama and Matsuki in preparation). Also, both RAD-seq and MIG-seq protocols are sufficiently generic for outsourcing. For future large-scale implementation of genetic optimization, botanical gardens without molecular facilities can choose to outsource the NGS library preparation and sequencing to private companies.

Aglaia teysmanniana: a pilot study for genetic optimization

A pilot study was initiated to test the genetic optimization protocol. Creating an *ex-situ* collection of rare and threatened species is a challenging task, in terms of locating the individuals or populations, and understanding the phenology (for fruit or seed collection) and germination requirements. In addition, there is a prolonged waiting period for fruiting and seed germination prior to transplantation. Therefore, the focal species in the pilot study has to

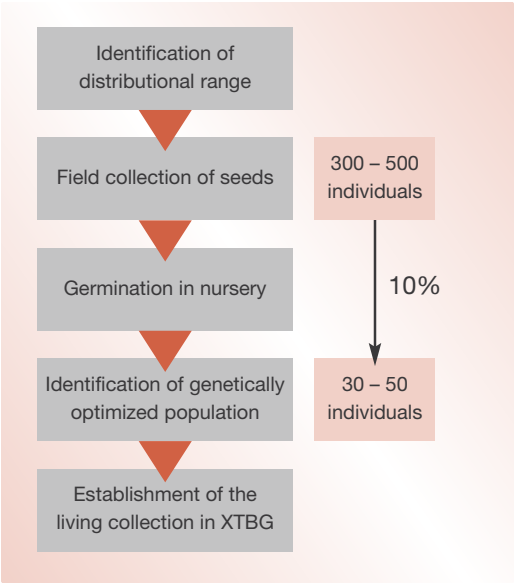


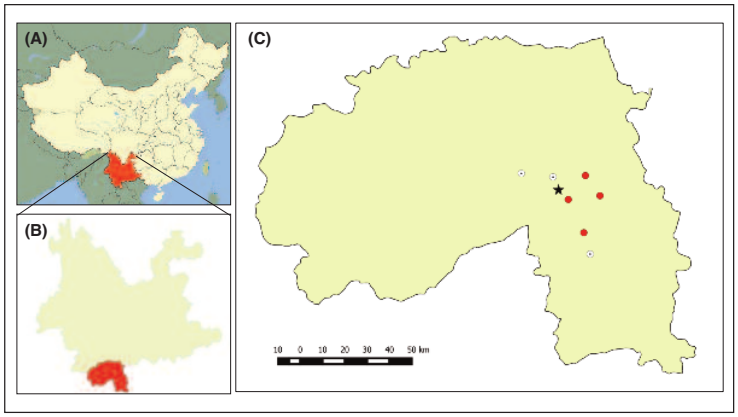
Figure 2: A schematic diagram of the workflow for genetic optimization

have (1) known and accessible populations, (2) known phenology, and (3) fairly simple and rapid germination.

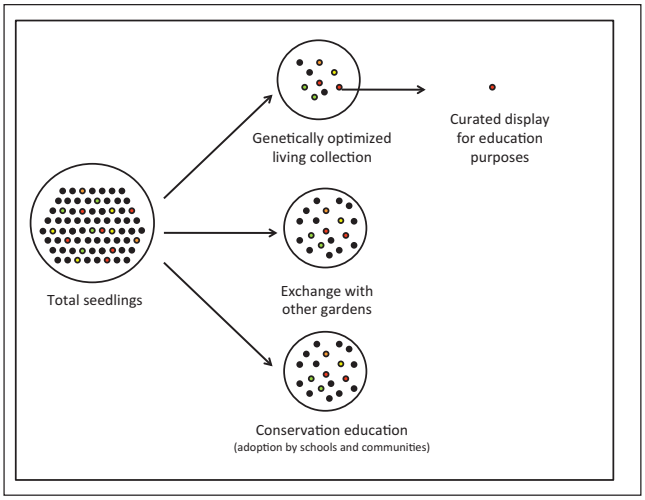
Aglaia teysmanniana (Meliaceae) is an ideal focal species for the pilot study. The seedlings are easy to identify in the wild and can be found in clusters near to the mother tree. Collecting seedlings from the wild allowed us to circumvent the phonological and germination challenges of sample collection. The species is distributed across most of Southeast Asia, including Indonesia, Malaysia, the Philippines, and Thailand. In China, it is found only in South and Southeast Yunnan, and is largely restricted to limestone forests. *Aglaia teysmanniana* is listed as Near Threatened on the IUCN Red List and Vulnerable on the Zero Extinction Project assessment in Xishuangbanna.

We collected 418 seedlings from seven populations in four locations in Xishuangbanna (Fig. 3). The GPS coordinate of the seedling patches were recorded, leaf samples of potential

Field collection of Aglaia teysmanniana *seedlings*



Map of the sampling locations. (A) The location of Yunnan province (in red) in China. (B) The location of Xishuangbanna prefecture (in red) in Yunnan province. (C) The locations of XTBG (black star), the limestone forest fragments (red) where Aglaia teysmanniana *seedlings were collected, and the limestone forest fragments (white) where* A. teysmanniana *was not found*



Future plan for transplanted seedlings

mother trees in the vicinity were collected, and the seedlings were transplanted to the XTBG nursery. Only 204 seedlings (49%) survived the transplantation process; root damage may be the cause of most of the mortality. The surviving seedlings will be genotyped with both RAD-seq and MIG-seq to evaluate the cost, time and labour requirements of both methods.

Future plans

The genotype of the seedlings (from both RAD-seq and MIG-seq) will be analyzed, and the most genetically diverse 10% of the total seedling population will be included in XTBG's living collection (Fig. 4). Among these, several individuals will be transplanted into the garden area that is open to the public for education purposes. The remaining seedlings will either be transplanted to other botanical gardens (as a backup living collection) or adopted by local schools and communities as part of XTBG's outreach program.

Upon completion of the pilot study, the most efficient and low-cost genotyping method of the two will be used to improve the *ex situ* conservation of other threatened tree species in the Zero Extinction Project's assessment, e.g. *Magnolia hypolampra, Cephalotaxus mannii* and *Goniothalamus cheliensis*.

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Transplanted seedlings in the XTBG nursery