Forest Genetic Monitoring in a Biodiversity Hotspot

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CENTRAL DOGMA OF CONSERVATION GENETICS

Genetic diversity is beneficial, hence worth preserving to the greatest extent

Conservation of biodiversity *ultimately depends on the conservation of genetic diversity and increasing genetic variance enhances the probability of* *population survival*.

Will the rate of adaptive evolution at least match the rate of environmental change, allowing populations to persist?

UNCERTAINTIES??? → **MONITORING!!!**

Conservation of Forest Genetic Resources

► *Maintain genetic diversity*
► *Designate protected areas*

Designating a forest as a protected area, does not shield it against climatic change

*Dr. Werner Kurz, Keynote Speaker, IUFRO2019*

RESEARCH

REVIEW SUMMARY

CLIMATE CHANGE

The human imperative of stabilizing global climate change at 1.5°C

O. Hoegh-Guldberg*, D. Jacob, M. Taylor, T. Guillén Bolaños, M. Bindi, S. Brown, I. A. Camilloni, A. Diedhiou, R. Djalante, K. Ebi, F. Engelbrecht, J. Guiot, Y. Hijioka, S. Mehrotra, C. W. Hope, A. J. Payne, H.-O. Pörtner, S. I. Seneviratne, A. Thomas, R. Warren, G. Zhou

Need for maintaining FGR greater than ever
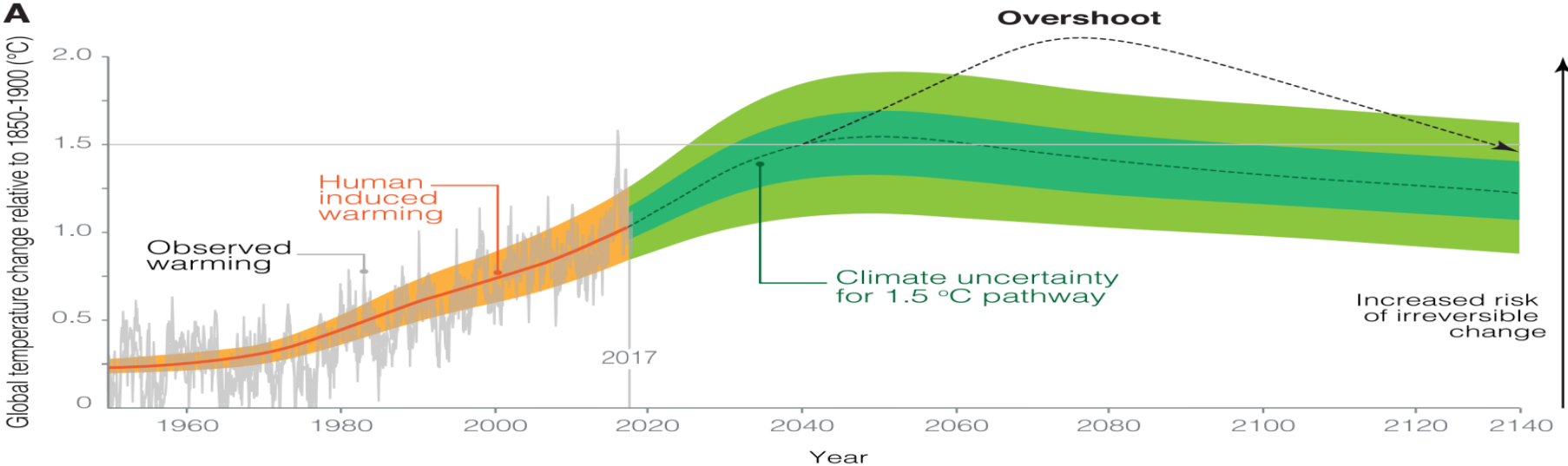
Hoegh-Guldberg *et al.*, *Science* **365**, 1263 (2019)   20 September 2019

Biosphere integrity may be more at risk in particular areas

Mediterranean: biodiversity hot spot, ~ 30% endemic species

Les ressources génétiques forestières dans le bassin méditerranéen

U. Alptekin, FA Aravanopoulos,
F. Asmar, M. Bariteau, A. Bentouati,
M. Benzyane, A. Derridj, F. Ducci,
F. Isik, A. Khaldi, M.L. Khouja,
E. Paoletti, P. Raddi,
S. Martin Albertos, H. Sbay,
G. Schiller, E. Teissier du Cros,
M.C. Varela, G.G. Vendramin

Une caractéristique essentielle des milieux méditerranéens sur le plan biologique est l'existence d'un fort impact anthropique dès l'apparition du climat méditerranéen, au Tardiglaciaire, ainsi qu'une **très forte diversité biologique** résultante d'une véritable co-évolution entre l'Homme et le milieu (CLEMENT, 1999)

Dans ces conditions, il faut appréhender très largement les ressources génétiques forestières méditerranéennes comme un patrimoine très précieux pour l'Humanité, de nature non seulement biologique mais aussi sociale et culturelle.

Recommandation

L'accentuation de la sécheresse, vraisemblablement liée au changement climatique,

Sud et à l'Est. La faible prise de conscience

Greece is a Biodiversity Hotspot

●One of the most species-rich European countries

● >7000 plant taxa (~ 20% endemics)

● 28% of the land base is included in the Natura 2000 Special Areas of Conservation

● Greek forest tree populations present significantly higher values of polymorphism and differentiation when compared to average values reported in the international literature

Aravanopoulos et al. 2019. Adv. Global Change Res. 65: 73-86.

●<u>Target</u>: "identify components of biological diversity" & "**<u>monitor</u>** through sampling & other techniques the components of biological diversity"

●<u>Focal area</u>: "Reducing the rate of loss of the components of biodiversity, including ... *genetic diversity*"

●<u>Indicator</u>: "Trends in *genetic diversity*"

Convention on Biological Diversity
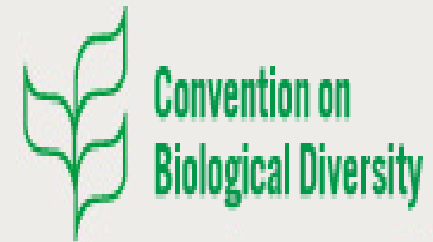
1992

AIM OF GENETIC MONITORING

*To assess the current status of genetic resources and quantify relevant changes **at a temporal scale** in light of preserving long-term adaptive evolutionary potential*

1st Lesson for Tropical Trees: Need to monitor in order to know how much diversity is there and if it is temporarily maintained.

- Study instrument.

- Early detection value
- Strategic tracing value
- Prognostic value.

- A means to secure the conservation of processes that maintain genetic variation & adaptive evolutionary potential in natural populations.

Genetic monitoring in natural perennial plant populations

F.A. Aravanopoulos

Abstract: Genetic monitoring, the quantification of temporal changes in population genetics and dynamics metrics generated by using appropriate parameters, constitutes a method with a prognostic value. Genetic monitoring has been recognized in several international agreements and documents, and can be an important tool for the protection of biodiversity. However, approaches developed so far for perennial plant species are rather cumbersome for practical use. It is proposed that perennial plant genetic monitoring should focus on keystone species of biological and economical importance, as well as rare or endangered species. In addition, genetic monitoring should concentrate on gene conservation units of such species, to be advanced in a dynamic gene conservation scheme. Three indicators are proposed for genetic monitoring based on a gene-ecological approach: natural selection, genetic drift, and a gene flow-mating system. These are evaluated based on three demographic (age and size class distribution, reproductive fitness, regeneration abundance) and four genetic (effective population size, allelic richness, latent genetic potential, outcrossing/actual inbreeding rate) parameters. Minimum sample sizes, critical levels of differences among parameters, and costs for temporal evaluation are proposed. The benefits of the immediate application of genetic monitoring are highlighted.

Key words: genetic monitoring, geneecological approach, indicators, gene conservation units.

Résumé : Le suivi génétique, soit la quantification des changements temporels de la génétique des populations et les mesures dynamiques générées par l'utilisation de paramètres appropriés, constitue une méthode valable pour poser des pronostiques. On a reconnu le suivi génétique dans plusieurs ententes et documents internationaux et il peut constituer un outil important pour la protection de la biodiversité. Cependant, les approches développées jusqu'ici pour les espèces végétales pérennes demeurent plutôt peu commodes pour un usage pratique. L'auteur propose que le suivi génétique des plantes pérennes se concentre sur des espèces indicatrices, d'importance biologique et économique, ainsi que d'espèces rares ou menacées. De plus, le suivi génétique devrait se concentrer sur les unités de conservation des gènes d'espèces dont le schème de conservation dynamique des gènes est avancé. L'auteur propose trois indicateurs pour le suivi génétique basé sur une approche génécologique : sélection naturelle, dérive génétique, et systèmes de flux des croisements. On évalue ceux-ci en se basant sur 3 paramètres démographiques (âge, distribution des classes de dimensions, adaptation reproductive, abondance de la régénération) et quatre paramètres génétiques (dimension réelle des populations, richesse en allèles, potentiel génétique latent, taux d'hétérocroisement/autocroisements actuels). On propose des dimensions minimales d'échantillons, des degrés critiques de différences parmi les paramètres et des coûts pour l'évaluation dans le temps.

Mots-clés : suivi génétique, approche génécologique, indicateurs, unités de conservation des gènes.

[Traduit par la Rédaction]

Introduction

The 20th century has been characterized by unprecedented scientific and technological advances, but at the same time, by an equally unprecedented exploitation and devastation of the natural environment. Almost 45% of the world's forests have already been depleted, and about 90% of this destruction has occurred in the past century (Anony-

mous 2010). At present, the species extinction rate is almost 1000× higher than historical levels, causing fear of large scale extinction, comparable with the last such extinction 65 million years ago. It has been estimated that it may take millions of years for biodiversity to recover from current anthropogenic extinctions (Kirchner and Weil 2000; Kirchner 2002). To counteract species extinction and biodiversity loss, the concept of biological conservation has been advanced. This refers to the science and management activities that ensure the survival of the maximum diversity of species, and the maintenance of genetic variation within species (Allaby 2006).

For almost two decades, the United Nation's Convention on Biological Diversity (CBD) has been the most important international political instrument dealing with issues of bio-

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Conservation and Monitoring of Tree Genetic Resources in Temperate Forests

Filippos A. (Phil) Aravanopoulos [1]

Abstract In the era of predicted significant environmental change, genetic conservation and monitoring of long-lived forest trees are of paramount importance for the survival of natural populations. Genetic conservation aims to protect and preserve genetic variation, vital for the maintenance of adaptive potential within populations and species. This paper discusses the advances made in gene conservation and genetic monitoring approaches in particular that aim to put into practise a concept that was developed more than 25 years ago, but rarely put into practise. Genetic monitoring, the quantification of temporal changes in population genetic variation and structure, elucidates processes that maintain genetic variation in natural populations, introduces prognosis and helps define tools for the management of forest genetic resources. Based on the geneecological approach, proposed indicators reflect the assessment of genetic variation, genetic drift, gene flow (mating system) and natural selection. Indicators are evaluated in contemporary monitoring schemes by 7–11 verifiers. Genomic data are expected to increase the precision of estimates of adaptive genetic potential and of population genetic parameters due to the significantly higher number of markers assayed. The transition from genetic to genomic monitoring should provide an enhanced potential for disentangling natural selection from demography, and deciphering the association of genetic variation to environmental gradients. In the future, genetic monitoring will be more limited by time-consuming procedures and funding constraints in the assessment of demographic parameters, as well as by conceptual and analytical weaknesses of biostatistical tools, than by genotyping needs and the amount of available sequence data. Its important contribution to applied forest management can be foreseen.

Keywords Conservation genetics · Genetic monitoring · Conservation genomics · Genomic monitoring · Forest trees

Introduction

In the past 300 years, the world has lost more than 700 million ha of tropical forest and more than 300 million ha of temperate forest, mainly due to anthropogenic impacts. Large forest destruction started during the Holocene and continued to the present day. In ancient Greece for instance, van Andel et al. [1] found repeated episodes of deforestation followed by soil erosion since the late Neolithic period. It is estimated that about 45 % of world forests have been lost, and about 90 % of this loss has occurred since the start of the twentieth century [2]. Continual forest destruction has a profound effect on biodiversity loss. Today, the rate of population extinction and species loss may be 1000× higher than background levels [2].

Despite these losses, trees still constitute more than 80 % of continental biomass and forests harbour more than 50 % of terrestrial biodiversity. Forests continue to offer a sustainable source of primary production that includes wood, paper, tree-crop foods, pharmaceuticals, bioenergy, as well as a multitude of ecological services. Forests preserve biodiversity and the genetic heritage of natural ecosystems and play a key role in carbon sequestration and storage, climate regulation, production and preservation of water quality, soil protection and erosion control. A large number of trees are keystone species for many natural ecosystems and form a cornerstone for the

How to Monitor – What to Measure?

- Proposed conceptual framework: the *geneecological approach*.

- The major forces of evolution at microscale are the effects of *natural selection* (that can lead to differentiation associated with local adaptation) and *genetic drift* (that can lead to differentiation associated with stochastic changes and genetic erosion), mediated by the action of *gene flow* (that can lead to genetic homogenization).
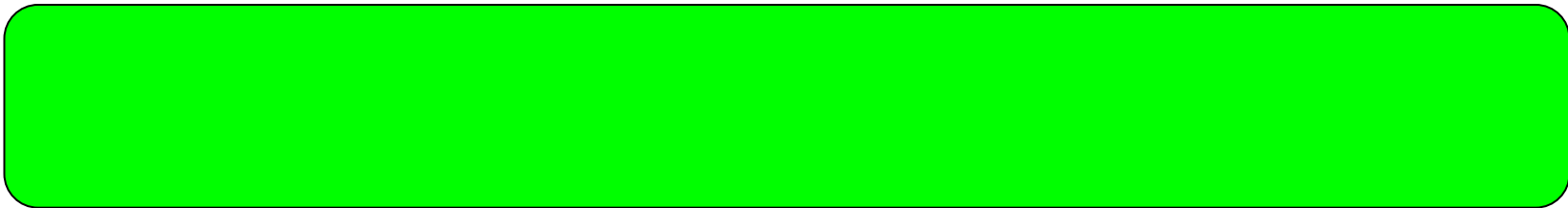
Indicator	Verifier	1-2 Y	5 Y	10-15 Y
Selection	age/size class distribution			√
	reproductive fitness – mast years (% of filled seeds, % of germination)		√	
	regeneration abundance		√	
	mortality / fructification	√		
	F_{ST} outlier analysis			√
Genetic drift	genetic diversity (allele/genotype frequencies,, N_A, P, H_E, H_O,, F_{IS}, F_{ST})			√
	effective population size (N_E)			√
	allelic richness			√
	latent genetic potential			√
Gene flow	outcrossing /actual Inbreeding rate			√

Aravanopoulos 2011, 2016

Temporal scale of Genetic Monitoring
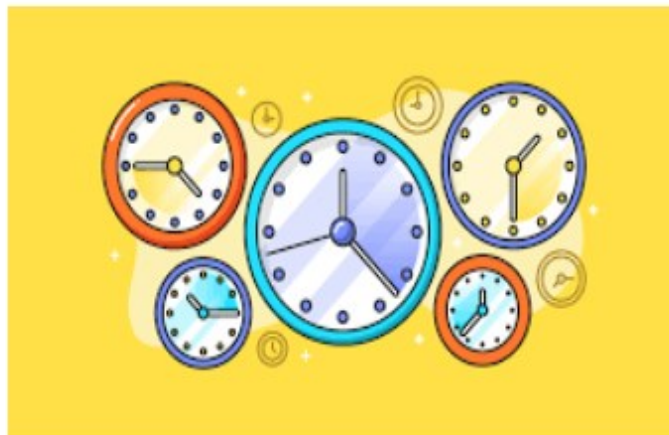
1st Genetic Marker Assessment

2nd Genetic Marker Assessment

- **Baseline data**   **5y**   **10y**   **15y**
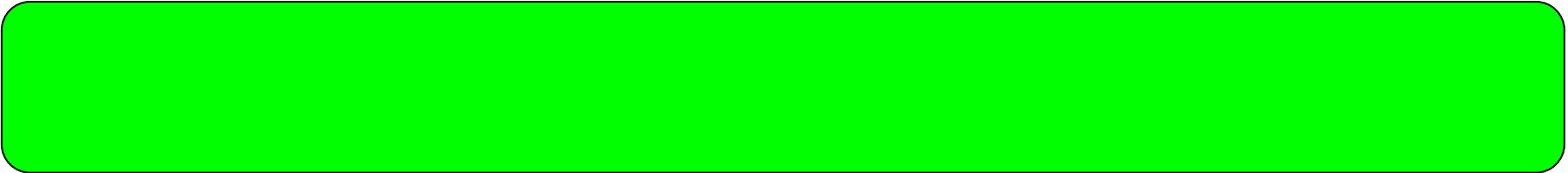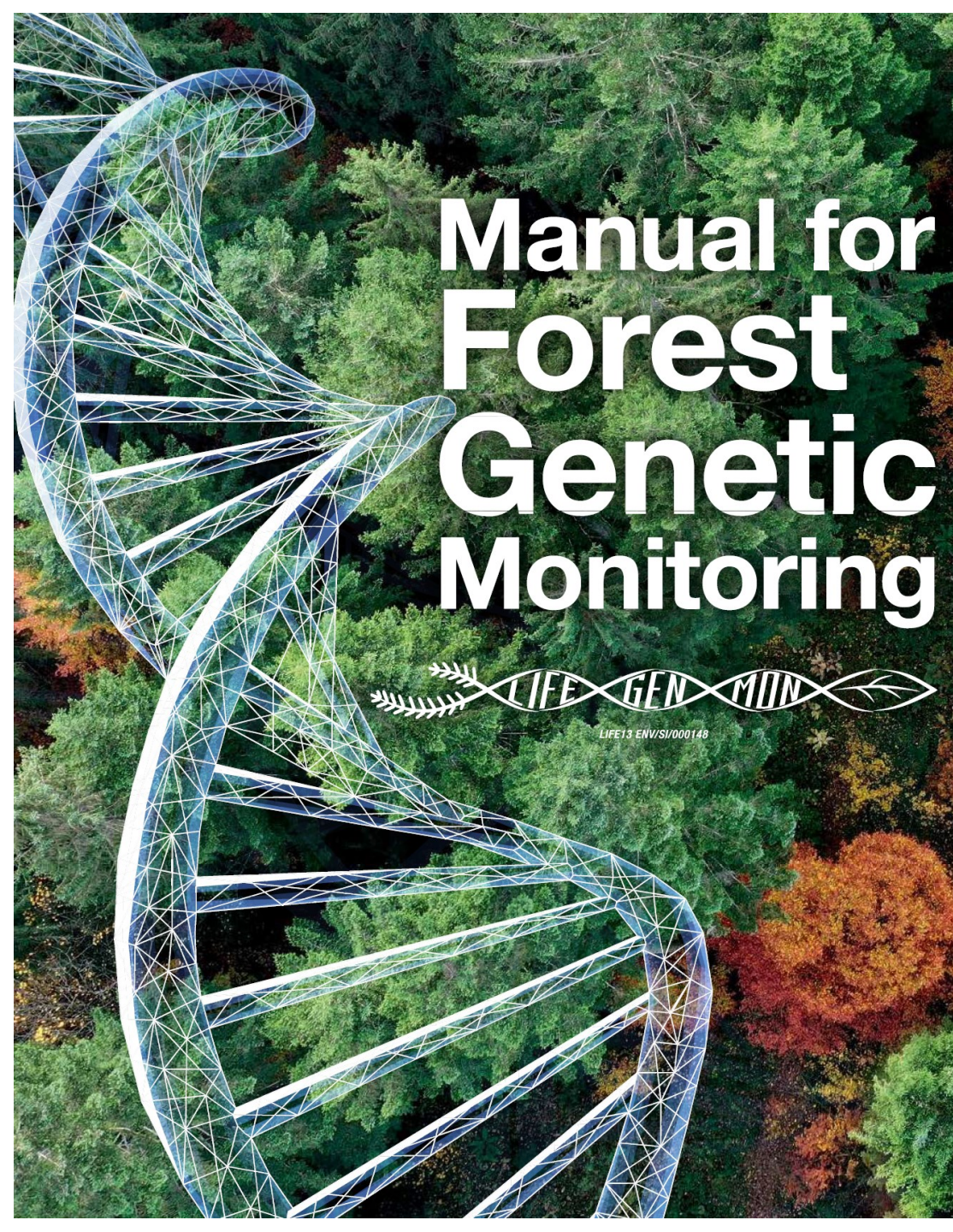
Indicator	Verifier	Critical Difference
Selection	age/size class distribution	2 standard errors
	reproductive fitness – mast years (% of filled seeds, % of germination)	> 50 %
	regeneration abundance	> 50 %
	mortality / fructification	> 50 %
	F_{ST} outlier analysis	> 25 %
Genetic drift	genetic diversity (allele/genotype frequencies,, N_A, P, H_E, H_O,, F_{IS}, F_{ST})	> 25 %
	effective population size (N_E)	< 50 (absolute value)
	allelic richness	> 25 %
	latent genetic potential	> 25 %
Gene flow	outcrossing /actual Inbreeding rate	>10 %

Aravanopoulos 2011, 2016

- **Plot size**: 4 ha & $N_{reproducing\ plants} \geq 50$ ($N_E \geq 50$)
- **Sample sizes**:
  - $N_{reproducing\ plants} \geq 50$
  - $N_{loci} \geq 100$ (SNP) (≥ 20 SSR)
  - $N_{seeds} \geq 1000$ (for Indicator-I)
  - $N_{seeds} \geq 300$ (for Indicator-II genetic analysis out of the Indicator-I sample)
- **Number of populations** : evaluation of ≥ 10 populations (GCUs) / species (preferable).
- **Temporal frequency**: one evaluation / 10-15 Y

Aravanopoulos 2011, 2016

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Manual for Forest Genetic Monitoring

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Table. 4.1: List of indicators and verifiers/background information for forest genetic monitoring at the basic, standard, and advanced levels, X: level at which a certain verifier is recorded, V: verifier, BI: background information.

Indicator	Verifier name	Type	Basic	Standard	Advanced
Selection	Mortality / Survival	V	X	X	X
	NR abundance	V	X	X	X
	Flowering	V	X	X	X
	Fructification	V	X	X	X
	% filled seeds	V			X
	% germination	V			X
	Crown dieback (ash)	BI	X	X	X
	Sex ratio (dioecious species)	BI		X	X
	DBH class distribution	BI		X	X
	Height class distribution	BI		X	X
	Flowering synchronisation	BI			X
	Budburst	BI		X	X
	Senescence	BI		X	X
Genetic variation	Allele frequencies	V		X	X
	Latent genetic potential	V		X	X
	Inbreeding coefficient	V		X	X
	Effective population size	V		X	X
	Allelic richness	V		X	X
	Linkage disequilibrium	V		X	X
	Interspecific hybridisation *	BI		X	X
	Multiplicity	BI		X	X
	F-analysis outlier test	BI		X	X
Gene flow / mating system	Gene flow	V			X
	Multi-locus population outcrossing rate	V			X
	Actual inbreeding rate	V			X
	Effective number of pollen donors	BI			X
	Biparental inbreeding	BI			X

* Hybridising species only

Bajc M, Aravanopoulos FA, Westergren M et al. (eds) 2020. Manual for Forest Genetic Monitoring. Silva Slovenica Publ., Ljubljana.

Table 8.1: A set of questions that can be answered by a given level of forest genetic monitoring.

Question	Forest genetic monitoring level		
	Basic	Standard	Advanced
Is fructification frequent enough (as expected for a given tree species)?	X	X	X
Is fructification abundant enough (as expected for a given tree species)?	X	X	X
Is there unexpected mortality that may cause population decline?	X	X	X
Is there enough natural regeneration to maintain evolutionary potential?	X	X	X
Is the effective population size large enough to sustain evolutionary potential?		X	X
Is the population undergoing long-term decline as estimated by the demographic models?		X	X
Is the variability in the population (genetic – molecular markers, phenotypic – trait variation) large enough to maintain evolutionary potential?		X	X
What is the reason for low regeneration abundance (not enough flowering, lack of synchronisation between male and female flowering, seed abortion, or lack of germination)?			X
Is there high inbreeding in a population? Is it increasing?			X
Can the gene flow from other populations counteract the decline of the monitored population?			X
Does the population have the potential to overcome the challenges imposed by the environment?			X

Bajc M, Aravanopoulos FA, Westergren M et al. (eds) 2020. Manual for Forest Genetic Monitoring. Silva Slovenica Publ., Ljubljana.

Forest Genetic Monitoring in Greece

Data

- Species involved: *Abies borissi regis, Castanea sativa, Fagus sylvatica, Quercus petraea, Quercus robur, Pinus nigra and Prunus avium.*

- Use of 11-26 SSR loci in different cohorts (mature and juvenile trees).

- Average time internal between assessments is 12.9 years.

Initial results

- *A. borissi regis* and *Fagus sylvatica* indicate maintenance of genetic diversity over time, but a slight drop of Ne in the juvenile cohort of hybrid fir compared to the mature trees.

Perspectives for Genetic Monitoring in Applied Forestry

- ***Genetic monitoring is paramount for the evaluation of forest genetic resources***; it forms an invaluable tool for future ecosystem protection & sustainable management. ***This is particularly important for ecosystems that are already exposed to climatic change.***

- The development of time series data for ***genetic monitoring has been pending*** for a long time ***in temperate forests.***

- The genetic monitoring proposed is a comprehensive and unified scheme that can be ***globally*** applied.

- Its application presents a twofold benefit: ***enhancement of genetic conservation and risk assessment based on prognosis for all forests***.

Throne of Zeus Peak, Mt. Olympus, Greece

Thank you for your attention

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