Genetic monitoring: theory, approach and value for conservation

Filippos A. (Phil) Aravanopoulos

Professor

- *Faculty of Agriculture, Forestry & Natural Environment, Aristotle University of Thessaloniki, Greece*
- *Full Member Hellenic Agricultural Academy, Chair of Forest Science*

Genetic Monitoring Training School 2022

Response of Forests to Environmental Change

SPECIES ATTRIBUTES

- Species ***sensitivity*** to environmental change.
- Species ***adaptive capacity***.
- Species ***exposure*** to climatic change.

SPECIES RESPONSE TO CHANGE

- Plant species will ***acclimate*** to shifting environmental conditions *via phenotypic plasticity*, developing and expressing particular traits in response to local environmental conditions.
- Over the longer term, tree species may ***disperse*** *via gene flow* to more favorable sites, potentially over long distances.
- Under strong environmental change species via *genic selection* may also ***adapt***, or ***become locally extinct***.

CENTRAL DOGMA OF CONSERVATION GENETICS

Genetic variability 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**.*

- Target: “identify components of biological diversity” & “***monitor*** through sampling & other techniques the components of biological diversity”

- Focal area: “Reducing the rate of loss of the components of biodiversity, including … *genetic diversity*”
- Indicator: “Trends in *genetic diversity*”

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

A Digression to Formality: Genetic Monitoring Definitions

- *quantification of **temporal changes** in population genomics and dynamics metrics*

- *dynamics of **transition** from the present to the future genetic status of a population such as a forest stand*

- ***tracking through time** to estimate demographic and/or population genetic parameters in order to infer whether adaptive changes are occurring*

The Genetic Monitoring Objective

- To assess the evolutionary potential and response of a species to temporal environmental change, starting with current status of its genetic resources evaluated with a set of criteria, indicators and verifiers.

- Study instrument.
- Early detection / Prognostic value.
- A means to secure the conservation of processes that maintain genetic variation & adaptive evolutionary processes in natural populations.

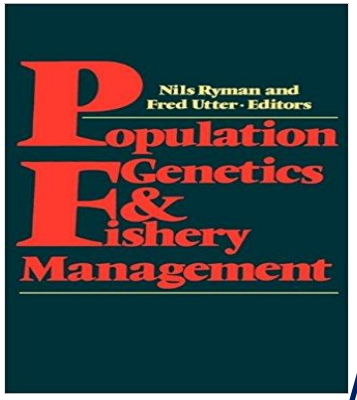
Criteria, Indicators & Verifiers

- ***Criterion**: something that is judged by (but without being a direct measure itself).*

- ***Indicator**: any component or process within a ecosystem described and used as a sign for the sustainability of a resource.*

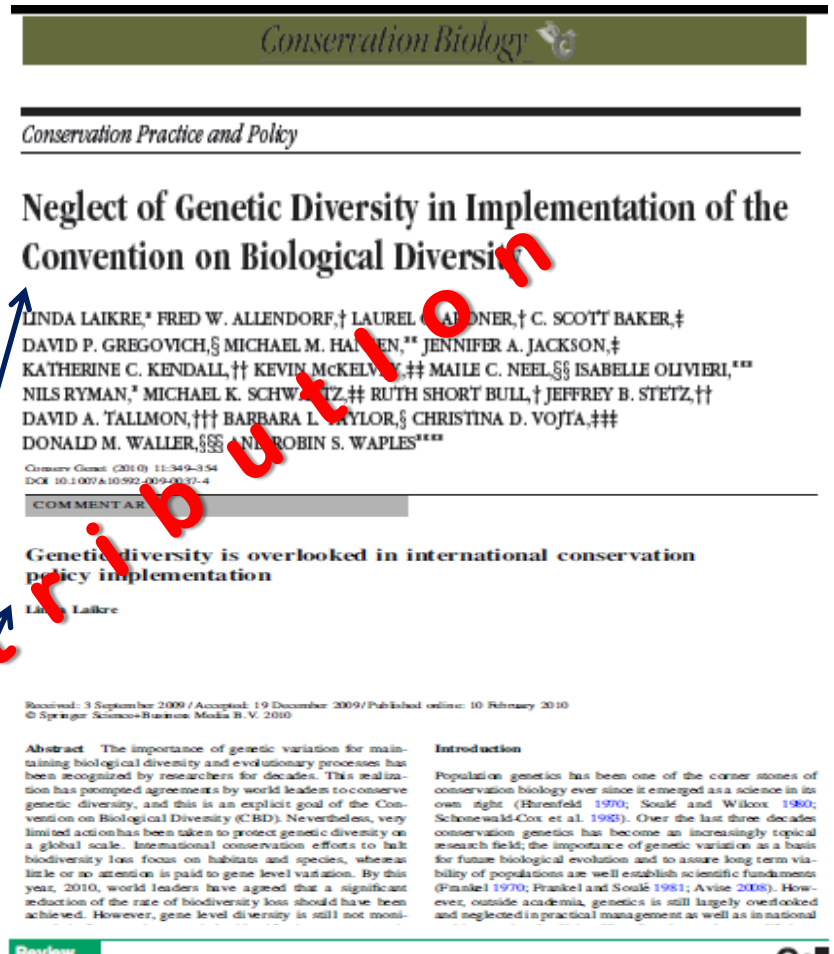
- ***Verifier**: a parameter needed to measure any component or process within the ecosystem in order to assess an indicator.*

The Genetic Monitoring debate



► Paper by Fred Allendorf and Nils Ryman (1987). Genetic management of hatchery stocks. *In* Population genetics and fishery management. *Edited by* N. Ryman and F.M. Utter. Univ. Washington Press, Seattle, pp. 141-159.

► Discussion-proposals have intensified in the last decade (Laikre et al. 2009, 2010a, b).


Conservation Biology

Conservation Practice and Policy

Neglect of Genetic Diversity in Implementation of the Convention on Biological Diversity

LINDA LAIKRE,* FRED W. ALLENDORF,† LAUREL C. [illegible]ONER,† C. SCOTT BAKER,‡ DAVID P. GREGOVICH,§ MICHAEL M. HANSEN,** JENNIFER A. JACKSON,‡ KATHERINE C. KENDALL,†† KEVIN McKELVEY,‡‡ MAILE C. NEEL,§§ ISABELLE OLIVIERI,*** NILS RYMAN,* MICHAEL K. SCHWARTZ,‡‡ RUTH SHORT BULL,† JEFFREY B. STETZ,†† DAVID A. TALLMON,††† BARBARA L. TAYLOR,§ CHRISTINA D. VOJTA,‡‡‡ DONALD M. WALLER,§§§ AND ROBIN S. WAPLES***

Conserv Genet (2010) 11:349–354
DOI 10.1007/s10592-009-0037-4

COMMENTARY

Genetic diversity is overlooked in international conservation policy implementation

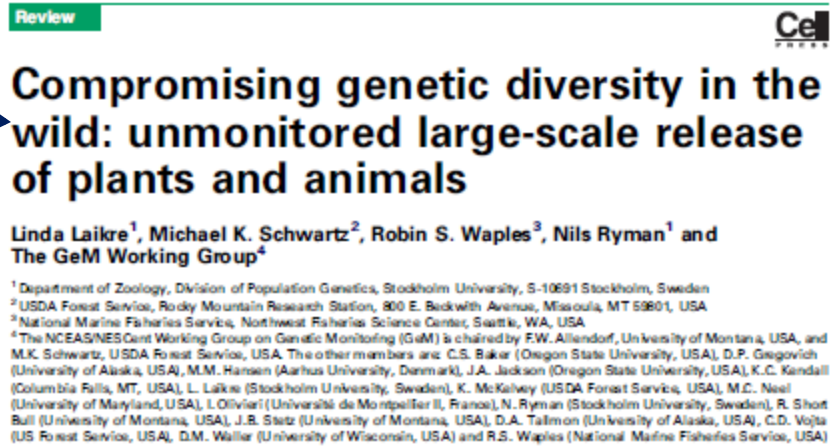
Linda Laikre

Received: 3 September 2009 / Accepted: 19 December 2009 / Published online: 10 February 2010
© Springer Science+Business Media B.V. 2010

Abstract The importance of genetic variation for maintaining biological diversity and evolutionary processes has been recognized by researchers for decades. This realization has prompted agreements by world leaders to conserve genetic diversity, and this is an explicit goal of the Convention on Biological Diversity (CBD). Nevertheless, very limited action has been taken to protect genetic diversity on a global scale. International conservation efforts to halt biodiversity loss focus on habitats and species, whereas little or no attention is paid to gene level variation. By this year, 2010, world leaders have agreed that a significant reduction of the rate of biodiversity loss should have been achieved. However, gene level diversity is still not moni-

Introduction

Population genetics has been one of the corner stones of conservation biology ever since it emerged as a science in its own right (Ehrenfeld 1970; Soulé and Wilcox 1980; Schonewald-Cox et al. 1983). Over the last three decades conservation genetics has become an increasingly topical research field; the importance of genetic variation as a basis for future biological evolution and to assure long term viability of populations are well establish scientific fundaments (Frankel 1970; Frankel and Soulé 1981; Avise 2008). However, outside academia, genetics is still largely overlooked and neglected in practical management as well as in national


Review

Cell Press

Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals

Linda Laikre[1], Michael K. Schwartz[2], Robin S. Waples[3], Nils Ryman[1] and The GeM Working Group[4]

[1] Department of Zoology, Division of Population Genetics, Stockholm University, S-10691 Stockholm, Sweden
[2] USDA Forest Service, Rocky Mountain Research Station, 800 E. Beckwith Avenue, Missoula, MT 59801, USA
[3] National Marine Fisheries Service, Northwest Fisheries Science Center, Seattle, WA, USA
[4] The NCEAS/NESCent Working Group on Genetic Monitoring (GeM) is chaired by F.W. Allendorf, University of Montana, USA, and M.K. Schwartz, USDA Forest Service, USA. The other members are: C.S. Baker (Oregon State University, USA), D.P. Gregovich (University of Alaska, USA), M.M. Hansen (Aarhus University, Denmark), J.A. Jackson (Oregon State University, USA), K.C. Kendall (Columbia Falls, MT, USA), L. Laikre (Stockholm University, Sweden), K. McKelvey (USDA Forest Service, USA), M.C. Neel (University of Maryland, USA), I. Olivieri (Université de Montpellier II, France), N. Ryman (Stockholm University, Sweden), R. Short Bull (University of Montana, USA), J.B. Stetz (University of Montana, USA), D.A. Tallmon (University of Alaska, USA), C.D. Vojta (US Forest Service, USA), D.M. Waller (University of Wisconsin, USA) and R.S. Waples (National Marine Fisheries Service, USA)

Indicators	Verifiers (genetic, demographic)	References
•levels of genetic variation, •selection, •gene migration, •genetic drift, •mating system, •hybridiza-tion •population structure, •population vital rates	•gene/genotype frequencies, •genotypic/allelic diversity, •gene flow, •population differentiation, •outcrossing/inbreeding rate, •P, •A, •N_E •N_A, •F_{IS}, •F_{ST}, •H_E •H_O, •adaptively significant traits in common garden experiments, •no. of potential parents, •phenotypic frequency distribution, •age class distribution, •regeneration, •pollen dispersal, •seed dispersal, •physical isolation by distance, •spatial aggregation of mating types, •sex ratios, •pollinator abundance, •parental population density, •proportion of filled seeds, •germination percentage, •fertility, •fructification	Allendorf *et al.* 2008; Anonymous 2006; Aravanopoulos 2011; Bariteau 2003; Granke *et al.* 2009; Graudal & Kjaer 2006; Hansen et al 2012; Konnert et al. 2011; Kuparinen & Merila 2007; Laikre *et al.* 2008; McKinnel 2002; Namkoong *et al.* 1996, 2002; Schoen *et al.* 2008; Schwartz *et al.* 2006

Evaluation of Indicators and Verifiers used thus far in Genetic Monitoring

- Numerous within a single scheme, or difficult to assess (time, expertise & financial elements)

- Indicators

- Verifiers

- Very general in order to be directly evaluated.
- Dependant on extensive data collection, or meta-analysis, in advance of application.

COMMENTARY / COMMENTAIRE

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, geneecologi

Résumé : Le suivi génétique, soit la quantif
sures dynamiques générées par l'utilisation d
nostiques. On a reconnu le suivi génétique d
outil important pour la protection de la biodi
tales pérennes demeurent plutôt peu commo
tes pérennes se concentre sur des espèces in
ou menacées. De plus, le suivi génétique de
schème de conservation dynamique des gèn
sur une approche génécologique : sélection
ceux-ci en se basant sur 3 paramètres démog
tive, abondance de la régénération) et quatre
potentiel génétique latent, taux d'hétérocrois
d'échantillons, des degrés critiques de différ

Mots-clés : suivi génétique, approche généco

[Traduit par la Rédaction]

Table 1. Parameters for the evaluation of temporal changes in the genetic monitoring of perennial plant gene conservation units.

Indicator	Metric trait	Genetic marker	Parameter	Critical difference between assessments
Selection	√		Age and size class distribution	Two standard errors
	√		Reproductive fitness: percentage of filled seeds and percentage of germination	≥50%
	√		Regeneration abundance	≥50%
Genetic drift		√	Effective population size	N<50 (absolute value)
			Allelic richness	≥25%
			Latent genetic potential	≥25%
Gene flow		√	Outcrossing/actual inbreeding rate	>10%

Introduction

The 20th century has been characterized by un
dented scientific and technological advances, but
same time, by an equally unprecedented exploitatio
devastation of the natural environment. Almost 45%
world's forests have already been depleted, and abou
of this destruction has occurred in the past century (A

Received 22 May 2010. Accepted 3 December 2010. Publ
on the NRC Research Press Web site at botany.nrc.ca on
14 January 2011.

F.A. Aravanopoulos. Laboratory of Forest Genetics and Forest Tree Breeding, Faculty of Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 238, Thessaloniki, 54124, Greece (e-mail: aravanop@for.auth.gr).

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-

Selection of Species - Monitoring Areas - Monitoring Method

- What do we want to conserve and monitor?
- Focus on keystone/model or vulnerable species?
- Focus on most valuable, or most vulnerable protected areas/priority gene pools/gene conservation units?
- Which will be the monitoring method?

Species selection

- Genetic monitoring *may* focus on:
  - Keystone/model species of ecological / economic importance; target species (principal aim: *prevention*)
  - endangered and/or rare species (principal aim: *restoration*)
  - "distributional or ecological margins of the species' natural distribution (principal aim: *prevention/ restoration*)

Not of distribution

Selection of Study Areas and Monitoring Method

- Monitoring should start from protected areas, preferably gene conservation units advanced in a dynamic gene conservation scheme (***priority: gene pools***).
- The monitoring method should be as much as possible unified and applicable to all species (**priority: "*species-free*"**).
- Sampling should be easy and straightforward in monitoring areas that likely will be situated in rather remote areas (***priority: easy sampling***).
- A minimum set of measured parameters should be selected in order to provide an adequate assessment of the monitoring area status at temporal scales (***priority: minimum # of parameters***).

Indicators and Verifiers

- Considerations:
  - merits
  - advantages / disadvantages
  - restricted number
  - species-free
  - pan-European application
  - essential genetic information not to be compromised
  - ease / difficulty of verifier assessment
  - temporal nature of measurements
  - technical expertise requirements
  - financial considerations
  - indicator interdependence / independence

Criteria

- One overall *criterion:*
  - ► *maintenance of genetic variation and adaptive genetic potential in natural populations*

Aravanopoulos 2011, 2016

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).

Aravanopoulos 2011, 2016

Genomic Monitoring Parameters: Basic Indicators & Verifiers

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

Critical differences in genomic monitoring parameters at the evaluation of temporal changes:

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

Minimum Sample Size Considerations:

- ***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

ARISTOTLE UNIVERSITY OF THESSALONIKI

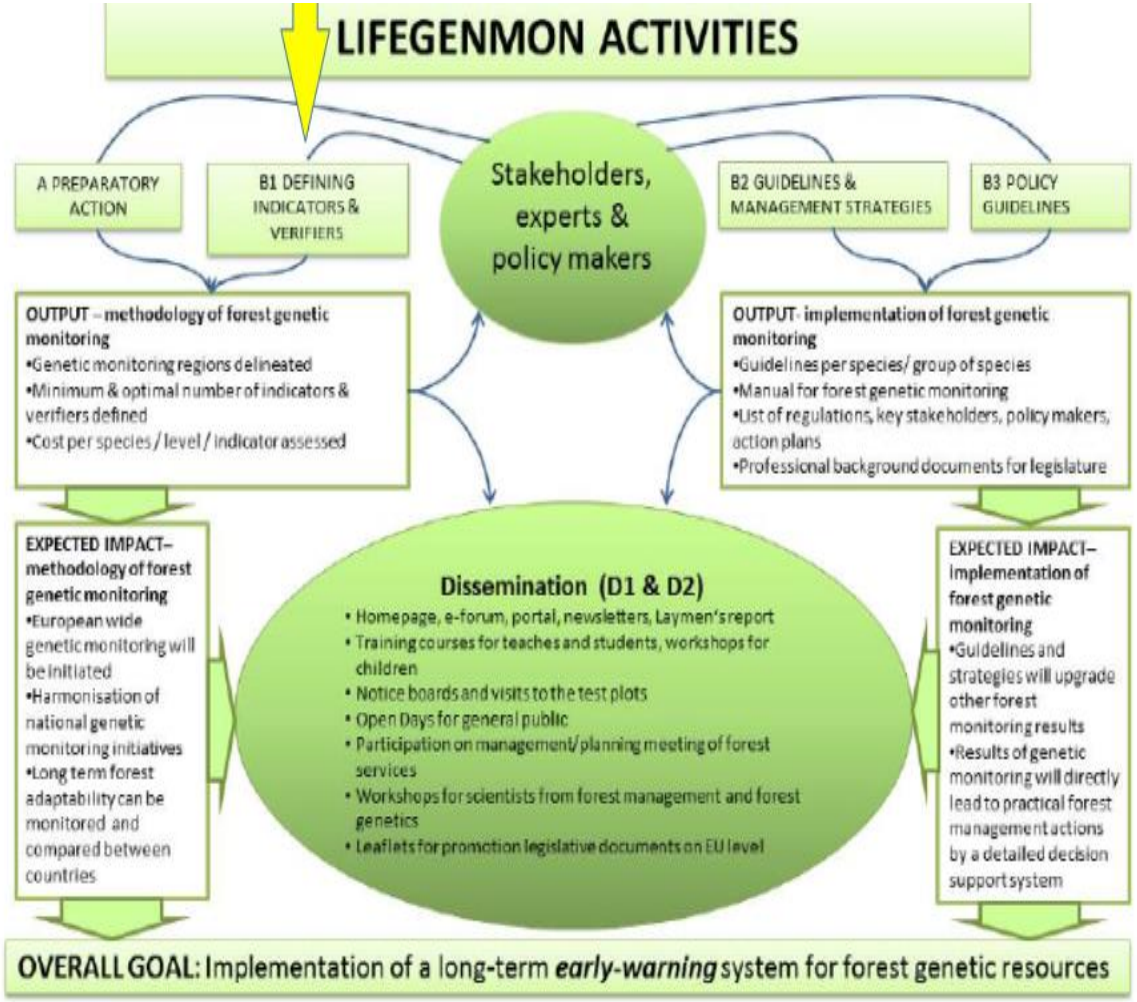
Feasibility: Indicators evaluated in monitoring schemes for forest trees

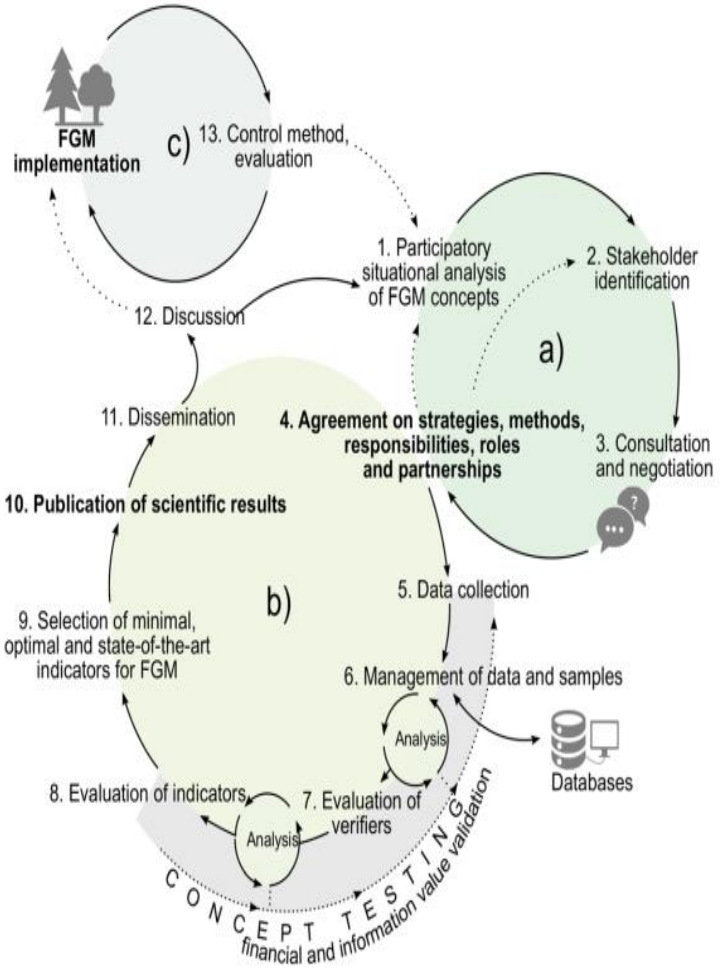
Indicators	*Citation*
3	Aravanopoulos 2011
18	Konnert *et al*. 2011 (German National System)
12	Schwartz *et al*. 2006
20	Namkoong *et al*. 1996 (FAO International System-I)
23	Namkoong *et al*. 2002 (FAO International System-II)
14	Geburek *et al.* 2010 (Austrian National System)

Feasibility: Verifiers evaluated in monitoring schemes for forest trees

Verifiers	*Citation*
7	Aravanopoulos 2011
18	Konnert *et al*. 2011 (German National System)
12	Schwartz *et al*. 2006
20	Namkoong *et al*. 1996 (FAO International System - I)
23	Namkoong *et al*. 2002 (FAO International System - II)
14	Geburek *et al.* 2010 (Austrian National System)

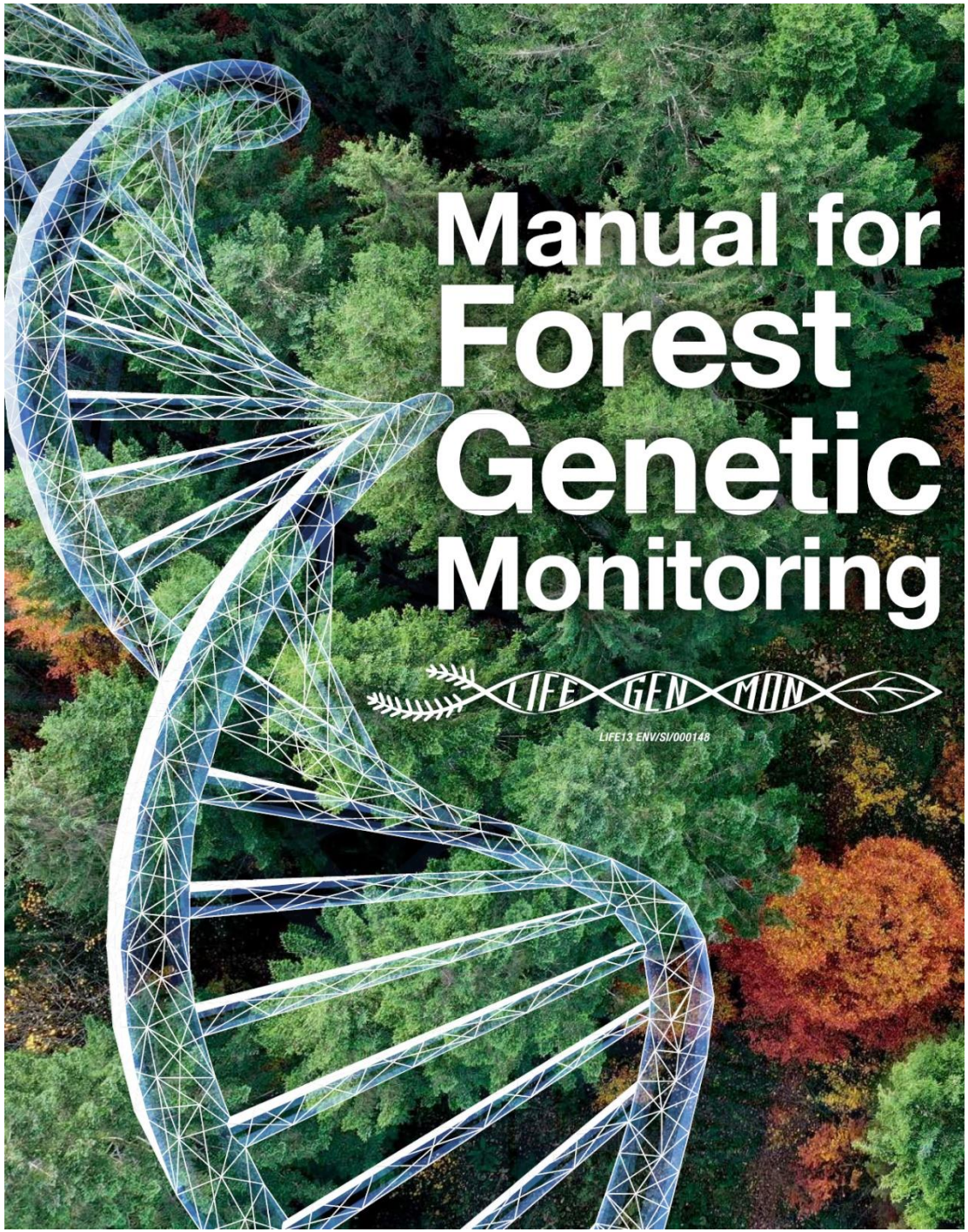
LIFE GEN MON





Finzgar et al. 2015, Fussi et al. 2016

The LIFEGENMON project: a <u>proof of principle exercise</u> for genetic monitoring.



Manual for Forest Genetic Monitoring

Editors

Marko Bajc

Filippos A. Aravanopoulos

Marjana Westergren

Barbara Fussi

Darius Kavaliauskas

Paraskevi Alizoti

Fotios Kiourtsis

Hojka Kraigher

AUTHORS

Filippos A. ARAVANOPOULOS[2], Marjana WESTERGREN[1], Barbara FUSSI[3], Darius KAVALIAUSKAS[3], Paraskevi ALIZOTI[2], Marko BAJC[1], Fotios KIOURTSIS[4], Monika KONNERT[3], Evangelia V. AVRAMIDOU[2,5], Dalibor BALLIAN[1,6], Evangelos BARBAS[2], Pavlos BEKIAROGLOU[4], Sándor BORDÁCS[7], Gregor BOŽIČ[1], Philip BRAILEY-JONES[1], Andrej BREZNIKAR[8], Pavlos CHASILIDIS[4], Rok DAMJANIĆ[1], Natalija DOVČ[1], Anna-Maria FARSAKOGLOU[2,9], Domen FINŽGAR[1,10], Nikitas FRAGKISKAKIS[4], Ioannis GANOPOULOS[2,11], Berthold HEINZE[12], Ermioni MALLIAROU[2], Georgios ROUSAKIS[4], Chryse SARVANI[4], Kristina SEVER[8], Nataša ŠIBANC[1], Nikolaos TOURVAS[2], Živan VESELIČ[8], Zvonimir VUJNOVIĆ[13], Peter ŽELEZNIK[1], Hojka KRAIGHER[1]

Proposed Options for Indicator and Verifier Assessment

- ***1st Option (Basic):*** Use of demographic data only. ***Indicator-I*** is fully evaluated.

- ***2nd Option (Standard):*** Use of demographic and genetic data. ***Indicator-I*** is fully evaluated (demographic verifiers). ***Indicator-II*** is fully evaluated (genetic verifiers using SSR and/or SNP genotyping).

- ***3rd Option (State-of-the-art):*** Use of demographic and genomic (NGS) data. ***Indicator-I*** is fully evaluated (demographic verifiers and signatures of selection provided by GWA of sequence data). ***Indicator-II*** is fully evaluated (genetic verifiers based on genomic (NGS) data).

Table. 4.2.1. 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
	Bud break	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

Verifier: Allelic richness

Indicator I: SELECTION

Allelic richness (Ar) is the total number of alleles in a population for a single locus averaged over all loci. Allelic richness is an estimate corrected by sample size (e.g. by rarefaction). It is used less commonly than heterozygosity as a genetic diversity measure, as it is harder to take into account the stochastic process of genetic drift for allelic richness. Nevertheless, allelic richness is considered to be a parameter that is more useful for gene conservation than allelic evenness (i.e. heterozygosity) (Brown and Schoen 1992; Rajora and Mosseler 2001, Aravanopoulos 2011). This verifier is associated with the use of microsatellite (SSR) genetic markers.

$$A = \left(\frac{1}{I}\right) A_i = \frac{\sum_{i=1}^{I} A_i}{I}$$

Number of alleles (A)

$$Ap = \sum_{i=1}^{I} Ap_i$$

Number of private alleles (Ap)

Indicator II: GENETIC VARIATION

Verifier: Latent genetic potential

Latent genetic potential (LGP) is an important genetic parameter that reflects the aptitude of a population to preserve adaptability under the multiplicity of changing environmental conditions (Stebbins and Hartl 1988; Bergmann et al. 1990). A population genetic analysis reveals its "operating genetic potential" (i.e. the part of its genetic composition which guarantees the survival of the population under present realised conditions, which is analogous to the effective number of alleles), while the remaining part in this context is currently "latent". This portion of genetic diversity is related to low frequency alleles in the population, which can nevertheless play a significant role for future adaptation under drastically changing environmental conditions, which can be of great importance for conservation practices (Aravanopoulos 2011, 2016). Therefore a change, and especially a reduction, of latent genetic potential may indicate a reduction of the overall adaptive capacity of the population. Latent genetic potential is computed as the difference between the total and effective number of alleles summed over all loci.

$$He_i = 1 - \sum p_i^2$$

Expected heterozygosity per locus (He_i)

$$Ae_i = \frac{1}{\left(1/He_i\right)} = \frac{1}{\sum p_i^2}$$

Effective number of alleles per locus (Ae_i)

$$LGP = \sum_{i}^{I} A_i - Ae_i$$

Latent genetic potential (LGP)

Verifier: Inbreeding coefficient

The inbreeding coefficient (F_{IS}) is the correlation of uniting gametes relative to gametes drawn at random from a subpopulation. It describes the variance within individuals, relative to their subpopulations. F_{IS} depends on the ratio of observed heterozygotes to the one expected under Hardy-Weinberg equilibrium, so it can also be seen as the reduction of heterozygosity of an individual compared to the subpopulation in the context of several (sub)populations that form the total (meta)population. An increase in inbreeding is evidently associated with a potential reduction of genetic diversity.

$$Ho_i = f(Bb)$$

Observed heterozygosity per locus (Ho)

$$He_i = 1 - \sum p_i^2$$

Expected heterozygosity per locus (He)

$$F = \sum_{i=1}^{I} \frac{Ho_i}{He_i} / I$$

Local F

Verifier: Effective population size

Effective population size (N_e) is one of the most (if not the most) important genetic parameters for genetic monitoring, as when it is small genetic drift becomes much more important than selection, and plays a paramount role in the evolutionary process. Therefore, a change, especially a reduction, in effective population size below acceptable threshold levels indicates the onset of genetic drift (as well as that of inbreeding). Hence it indicates both the onset of random and stochastic processes in the population and that of inbreeding and a potential reduction of genetic variation, which overall raise questions as to the future adaptive capacity of the population. Effective population size is defined as the number of individuals

$$Ne = \frac{1}{\frac{1}{T}\sum_{t=1}^{T} \frac{1}{N_t}}$$

Verifier: Linkage disequilibrium

Linkage disequilibrium (LD) is the non-random association of alleles at different loci in each populatic and is seen when the frequency of association of the different alleles at a locus is higher or lower th what would be expected if the loci were associated randomly (i.e. were independent) (Weir 1979). Linka disequilibrium can be affected both by evolutionary forces (see the Hardy-Weinberg equilibrium abo and demographic properties (population structure, asexual reproduction). For instance, linka disequilibrium will manifest itself or become more prominent in small populations, in populations unc strong evolutionary forces or under admixture. Therefore, linkage disequilibrium can be a powerful sig to denote underlying genetic and demographic processes in a population.

$$\bar{r}_d = \frac{\sum\sum cov_{i^1,i^2}}{\sum\sum\sqrt{var_{i^1} \cdot var_{i^2}}}$$

Indicator III: GENE FLOW / MATING SYSTEM

Verifier: Gene flow (Nm)

Gene flow is the exchange of genes through seed and pollen among populations that differ in genotypic frequencies. Gene flow is interceded by the mating system that mediates the recombination and assortment of genes between generations and determines the extent to which genes are exchanged among individuals, as well as immigration and emigration. It can be considered either beneficial or harmful from the point of view of a conservation genetics, forest genetic monitoring or tree breeding (Burczyk et al. 2004). Gene flow causes changes in the composition of the gene pool (allele frequencies) of the recipient population by incorporating alleles into its gene pool. The introduction of new alleles through gene flow increases genetic variability within the population and enables evolution and the combinations of traits (Encyclopaedia Britannica 2019, Mallet 1999, Burczyk et al. 2004, Aravanopoulos 2011). Gene flow is determined by the mating system that mediates the recombination and variety of genes between generations and determines the level to which genes are exchanged between individuals and populations (Aravanopoulos 2011). Gene flow measurement provides indirect information on the level of migration among subpopulations (Tibayrenc 2017).

$$Nm = \left(\frac{1 - F_{ST}}{4F_{ST}}\right)$$

Verifier: Multi-locus population outcrossing rate (tm)

The mating system is one of the key factors shaping population genetic structure (Hartl and Clark, 1989, Del Castillo and Trujillo 2008, Whitehead et al. 2018). Different mating systems influence the levels and dynamics of genetic diversity, effective population size and population differentiation, and overall it can affect population resilience and adaptation (Del Castillo and Trujillo 2008). Plant mating systems are usually defined by the mixed mating model, where one portion of seeds and ensuing plants are derived from various levels of inbreeding and the rest are derived from outcrossing at random (Ritland 2002). In particular, outcrossing promotes gene flow, homogenises populations, increases heterozygosity, and favours gametic linkage equilibrium (Del Castillo and Trujillo 2008). Outcrossing refers to the mating of genetically unrelated individuals and is the opposite of inbreeding (Aravanopoulos 2011). Multilocus outcrossing rate (tm) is an estimate of the proportion of outcrossed progeny produced by the population as a whole, in which outcrossing events include mating between relatives and unrelated individuals. A difference, e.g. an increase in the multilocus population outcrossing rate (tm), is an indication of maintenance if not of an increase of multilocus genetic variation, a result that will likely result in the maintenance of population adaptive capacity.

$$\hat{t}_m = 1 - \hat{s}_m$$

Multilocus estimation for individual outcrossing rate

$$\hat{s}_m = \frac{I_m - P_m^s}{P_m^s - P_m^t}$$

Multilocus estimation for individual selfing rate

$$Var(\hat{s}_m) = \frac{P_m^t(1 - P_m^t)}{(P_m^s - P_m^t)^2}$$

Variance of the multilocus individual selfing rate

Verifier: Actual inbreeding rate

The estimation of actual inbreeding rate (single locus and multilocus) is based on seed and genetic data (Rajora et al. 2000a, 2002; O' Connell et al. 2006). This is an important parameter as well, since, for example, an increase in the inbreeding rate may lead to allelic fixation and the reduction of population genetic diversity. The estimation of inbreeding rates can be marker-based, however as potential inbreeding depression may adversely affect seed development and germination, actual inbreeding rates are more reliable. Actual inbreeding rate is calculated by combining selfing estimates (1 – tm) from the mating systems analysis and seed-trait-based inbreeding estimates. It is the ratio of: [number of empty seeds per fruit + (number of filled seeds per fruit × selfing rate)] / [number of inbred seeds per fruit + number of filled seeds per fruit].

$$Actual\ Inbreeding\ Rate = \frac{B + C \cdot \hat{s}_m}{B + C}$$

Conclusions - Perspectives

- Genetic monitoring is paramount for the evaluation of forest genetic resources; it forms an invaluable tool for future ecosystem protection & sustainable management.
- The development of time series data for genetic monitoring has been pending for a long time.
- 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.

Conclusions - Perspectives

RECORD → ANALYSE → ALERT

GENETIC MONITORING ROLE

DOCUMENTARY → EXPLANATORY → PREDICTIVE

ARISTOTLE
UNIVERSITY
OF THESSALONIKI