

Forest Genetic Monitoring (FGM) plots selection, establishment and maintenance

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Manual for

Genetic Monitoring

VIEX GENXMIND

Forest

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Environmental monitoring - a group of systematic studies that reveal the state of the environment (EU JRC).

(https://joint-research-centre.ec.europa.eu/scientific-activities-z/environmentalmonitoring_en)

How does Forest Genetic Monitoring work?



FGM Implementation in DE

- The pilot implementation was based on the "Concept of a Genetic Monitoring for Forest Tree Species in the Federal Republic of Germany" (BLAG-FGR 2004).
- First national initiative implementation started in Germany in 2004 (Konnert et al. 2011) on two species European beech (*Fagus sylvatica*) and wild cherry (*Prunus avium*).

Tab. 2 - Overview of the sampling procedure to be carried out in the 3 sections of a monito - ring plot.

Developmental state	Intensive section including core section	Extensive section
Genetic Studies		
adult trees	all individuals present	additional individuals up to max. 300
natural regeneration	200 young plants representative	e for the overall plot
	4 clusters of natural regeneration comprising 50 individuals each	on -
beechnuts seeds	collecting per individual tree se	parately beechnuts from 20 adult trees
Quality structure of	beechnut seeds	
beechnuts seeds	seed mixture of the overall stan	d



Collection: IUFRO RG 7.01 (2010) - Antalya (Turkey) Adaptation of Forest Ecosystems to Air Pollution and Climate Change Guest Editors: Elena Paoletti, Yusuf Serengil

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Genetic monitoring in forests - early warning and controlling system for ecosystemic changes

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EUFORGEN, FGM and monitoring regions

 Aravanopoulos, F. A. et al. (2015). Development of genetic monitoring methods for genetic conservation units of forest trees in Europe. European Forest Genetic Resources Programme (EUFORGEN), Bioversity
 International, Rome, Italy. xvi+ 55 p.

https://www.euforgen.org/publications/ publication/genetic-monitoringmethods-for-genetic-conservation-unitsof-forest-trees-in-europe/



Genetic monitoring methods for genetic conservation units of forest trees in Europe



Genetic monitoring

methods for genetic

conservation units of

forest trees in Europe

Filippos Aravanopoulos, Mari Mette Tollefsruc Lars Graudal, Jarkko Koskela, Ralf Kätzel, Alvaro Soto, László Nagy, Andrej Pilipovic, Peter Zheley, Gregor Božić, and Genetic diversity is an essential element of tree species adaptation to climate change and other environmental changes. While several schemes have been set up during the past 30 years to monitor the health and biodiversity of forests in Europe, none of them expressly collects information about status and trends of genetic diversity. EUFORGEN has emphasized the importance of genetic monitoring as part of its previous activities and more recently, the Steering Committee established a working group to review genetic monitoring methods and to propose options for creating a pan-European genetic monitoring system for genetic conservation units of forest trees. This report presents the findings and recommendations of the working group.

Authors: Filippos Aravanopoulos; Mari Mette Tollefsrud; Lars Graudal; Jarkko Koskela; Ralf Kätzel; Alvaro Soto de Viana; László Nagy; Andrej Pilipović; Peter Zhelev Stoyanov; Gregor Bozič; Michele Bozzano

Principles and processes for selecting genetic monitoring units within monitoring regions (EUFORGEN WG on FGM)

- The goal of genetic monitoring is linked to the conservation of the long-term adaptive evolutionary potential. Therefore, when establishing acceptable limits to sampling, it is advisable to include all the ecological situations in which the species in question occurs.
- Accurate FGM of FGR requires the most representative sampling possible.
- However, limited resources (personnel, funds, data handling capacity) impose the necessity for a more restricted coverage of units in the sampling.
- Other available information regarding genetic diversity and phylogeographical patterns, phenology data, etc. must also be included in the decision-making process.
- FGM network (as a whole, including different model species) should involve all European countries and comprise a balanced selection of genetic monitoring units.

Species selection for FGM (EUFORGEN)

- EUFORGEN WG identified 14 "keystone" species) and one (1) "endangered" species (15 species in total) as a "pilot species" for FGM;
- Furthermore, after literature survey WG identified 14 species which have published genetic information on the species that could be of interest and usable for FGM

 Table 4. Relevant genetic markers and associated genetic information on the 14 keystone species

Species	nSSR genetic markers and genetic information	SNP genetic markers and genomic information
Abies alba	Cremer et al., 2006; Gomory et al., 2012; Vendramin et al., 1999	Mosca <i>et al.</i> , 2012a, b
Castanea sativa	Kremer et al., 2012	Marinoni et al., 2003
Fagus sylvatica	Jump, Hunt and Penuelas, 2007; Magri <i>et al.</i> , 2006; Lander <i>et al.</i> , 2011; Lefevre <i>et al.</i> , 2012	Seifert, Vornam and Finkeldey, 2012
Fraxinus excelsior	Gerard <i>et al.</i> , 2013; Heuertz <i>et al.</i> , 2004	http://www.ashgenome.org/ ; http://oadb.tsl. ac.uk/
Picea abies	Scotti et al., 2002a, b; Tollefsrud et al., 2009	Chen et al., 2012a, b; Heuertz et al., 2006; http://bfw.ac.at/rz/bfwcms2.web?dok=9020
Pinus brutia	Keys et al., 2000	_
Pinus cembra	Salzer et al., 2009	Mosca et al., 2012a, b
Pinus halepensis	Chagné <i>et al.</i> , 2004; Keys <i>et al.</i> , 2000; Troupin, Nathan and Vendramin, 2006	Grivet <i>et al.</i> , 2011
Pinus nigra	Gonzalez-Martinez et al., 2004	_
Pinus sylvestris	Garcia-Gil <i>et al.</i> , 2009; Soranzo, Provan and Powell, 1998	García-Gil, Mikkonen and Savolainen, 2003; Garcia-Gil <i>et al.</i> , 2009; Pyhäjärvi <i>et al.</i> , 2007; http://bfw.ac.at/rz/bfwcms2.web?dok=9020
Quercus petraea	Neophytou et al., 2010	Vornam et al., 2011
Populus nigra	Cervera <i>et al.</i> , 2001; Smulders <i>et al.</i> , 2008a, b	Chu <i>et al</i> ., 2009
Populus tremula	de Carvalho <i>et al</i> ., 2010; Hall <i>et al</i> ., 2007	Hall <i>et al.</i> , 2007
Sorbus torminalis	Hoebee et al., 2007	_

Aravanopoulos, F. A. et al. (2015)

Criteria for the selection of tree species for FGM (EUFORGEN)

For further species selection WG considered:

- (1) species having both SSR and SNP information available;
- (2) species distributed in the broad ecological categories defined on the basis of geographical distribution (wide or restricted distribution) and ecological appearance (stand-forming or scattered);
- (3) species that present genetic conservation units with predominantly N>50 trees;
- (4) species with a predominantly south-central and southern distribution, which can be considered as having greater vulnerability to CC;
- (5) species used by the relevant projects (e.g FORGER).
- (6) Based on these criteria, the working group proposes the following species: Abies alba, Castanea sativa, Quercus petraea, Picea abies, Pinus halepensis and Populus nigra.

FGM monitoring regions (EUFORGEN)

The working group tested three different approaches for identifying potential FGM regions at the pan-European level.

- **1.** Species distribution × environmental zone
- 2. Species distribution × country × environmental zone × stratum
- 3. Species distribution × grid option
- Although these approaches, focusing on quantitative background data, have proven not to be suitable.
- Testing directly contributed to the development of the approach that was finally chosen.

Criteria for the selection of the monitoring regions and the number of units per region (EUFORGEN)

- WG has agreed on the principles for identifying FGM regions;
- FGM regions have been identified and the potential number of genetic monitoring units proposed.
- While the working group has identified FGM regions and recommended the number of FGM units for each region, the final selection of genetic monitoring units will be the task of participating countries and their relevant authorities.



forest tree species investigated for the establishment of genetic monitoring regions **Keystone species** Abies alba Castanea sativa Fagus sylvatica Fraxinus excelsior Picea abies Pinus brutia Pinus cembra Pinus halepensis Pinus nigra Pinus sylvestris Populus nigra Populus tremula Quercus petraea Sorbus torminalis Endangered species at the pan-European level Ulmus laevis

Table 3. Keystone and endangered

Aravanopoulos, F. A. et al. (2015)

Criteria for the selection of the number of units per region (EUFORGEN)

- The monitoring units will be selected following an expert-based approach, defining the total number needed for each species and the most appropriate placement within the species distribution range. For the exact identification of the genetic monitoring unit, the following additional criteria should be used:
 - Population size: minimum 50 reproducing trees.
 - Unit size: minimum 4 ha for stand-forming species.



Specific criteria to be taken into account in the selection of genetic monitoring units (1)

Specific value

 Genetic monitoring units must be representative of the genetic resources for which the monitoring region was selected.

Multipurpose units

 It is advisable to concentrate monitoring efforts in multipurpose units. (Suitable for FGM of one or more species, included in permanent - intensive study plots, or intensive study sites of national or regional forest inventories (e.g. IPC; other networks; plots already established in national or European projects; etc.).

Specific criteria to be taken into account in the selection of genetic monitoring units (2)

Management criteria

- In GCU network, many different types of regeneration regimes as well as silvicultural and management techniques.
- It would be useful to assess the long-term influence of different large-scale management regimes on FGR.
- However, because of limited resources genetic monitoring units may focus on natural populations with minimal anthropogenic intervention.
- From a practical point of view, FGM units will be selected from existing conservation units.
- Need of commitment with owners and managers (and their formal agreement) to monitoring efforts.

Other criteria for FGM units selection: Size >4 ha (for stand-forming species, while for species with a scattered distribution the size will depend on the minimum required number of reproducing trees included in the plot) No of trees, Ownership, Conservation status and threats, Genetic uniqueness, etc.

Summary (EUFORGEN)

- 1. Target tree species selection
- 2. Species distribution and existing GCU's (EUFGIS)
- Representative coverage of environmental zones + spp.
 distribution + other relevant info = FGM regions
- 4. FGM units identification
- 5. FGM plot selection in certain FGM region
- 6. FGM design, size, no of trees, available data, etc.



LIFEGENMON project

Bajc, M., Aravanopoulos, F., Westergren, M., Fussi, B., Kavaliauskas, D., Alizoti, P., ... Kraigher, H. (Ur.). (2020). Manual for forest genetic monitoring. Ljubljana: Slovenian Forestry Institute, Silva Slovenica Publishing Centre.

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LIFEGENMON 2014-2021

One of the aim of project was to prepare FGM manual and guidelines for forest genetic monitoring

For two model tree species:

- Abies alba/Abies borisii-regis complex
- Fagus sylvatica

Additional five forest trees species:

- Fraxinus excelsior
- Pinus nigra
- Populus nigra
- Prunus avium
- Quercus petraea/robur complex
- For implementation of FGM at a national, regional and EU scale.



Illustrations by:		Marija Prelog	Fagus sylvatica L.
Metka Kladnik	Fraxinus excelsior L.	Klara Jager	Pinus nigra Arnold
Anja Rupar	Abies alba Mill.	Eva Margon	Quercus petraea (Matt.) Liebl. / Quercus robur L.
Teja Milavec	Prunus avium (L.) L.	Marina Gabor	Populus nigra L.

Monitoring regions should be delineated based on the following criteria:

- 1. Representative coverage of environmental zones;
- 2. Coverage of characterised races or ecotypes, inclusion of marginal and peripheral populations considering latitudinal, altitudinal and ecological margins, as well as leading and rear edge populations of the species distribution range;
- 3. Consideration of the distribution of **EUFORGEN gene conservation units** (EUFORGEN, <u>http://portal.eufgis.org/</u>), so that each **genetic monitoring region preferably includes at least one GCU as a genetic monitoring unit**, if the relevant requirements are met;
- 4. Known levels of **existing genetic structure and standing genetic variation** based on the results of genetic marker research;
- 5. Relevant results of provenance trials;
- 6. Expert knowledge on a country basis should be used to fine tune the locations of the delineated monitoring regions regarding the forest types, vitality, biodiversity and economic value of populations.
- 7. In the case of unclear or only partial results being available, expert opinion should be used.

Description of designation and maps of monitoring regions within LIFEGENMON

- Within LIFEGENMON, the delineation of FGM regions has been carried out by a joint data-driven and expert-based approach, in a NW to SE transect spanning from Bavaria to Greece.
- Seven priority species with contrasting biology, ecology and distributional properties were employed (Abies alba/A. borisii-regis complex, Fagus sylvatica, Fraxinus excelsior, Pinus nigra, Populus nigra, Prunus avium, and Quercus robur/Q. petraea complex).
- 6 to 10 monitoring regions per species/species complex were recognised.
- The monitoring regions as delineated within the LIFEGENMON project are valid for the transect from Bavaria to mainland Greece. If a wider, pan-European, area was assessed, the monitoring regions might be delineated differently.





Monitoring regions over the transect from Bavaria to Greece for Fagus sylvatica, Abies alba/ A. borisii regis, Fraxinus excelsior, Populus nigra, Pinus nigra, Prunus avium and Quercus robur/Q. petraea

Monitoring tree <i>spp.</i>	No. of FGM regions
Abies alba/A. borisii-regis complex	9
Fagus sylvatica,	10
Fraxinus excelsior	6
Pinus nigra	7
Populus nigra	8
Prunus avium	8
Quercus robur/Q. petraea complex	7





Monitoring regions for European beech (Fagus sylvatica)





Number of FGM plots per species

- It is recommended that at least one (1) forest genetic monitoring (FGM) plot be established per monitoring region;
- Monitoring regions are delineated per species or species complex
- If neighbouring countries share monitoring regions, international cooperation can reduce the total number of FGM plots across countries so that each monitoring region is represented by one FGM plot.



FGM plot selection (1)

- Priority for forest stands with high data density (e.g. genetic experimental plots, yield and growth permanent observation plots, NFI plots, etc.)
- Background data during selection (e.g. different biological and environmental situations, natural distribution range, climatic and environmental data, soil, vegetation, etc.)
- FGM plot should not have experienced clear-cutting in the recent history
- Exclusion of any clear-cutting under current and future management
- Presence of stand-level attributes paramount for genetic monitoring e.g. age of the stand / developmental phase
- Attributes linked to the ecological adaptation of the population to the site:
 - Fructification/Seeds availability
 - Presence and survival of natural regeneration



FGM plot selection (2)

Concordance of the site with EUFGIS requirements (sites should agree with the EUFGIS minimum requirements):

- 1. The units should have a **designated status** (e.g. gene conservation area, protected area, etc);
- 2. The units can be located in forests managed for multiple uses, protected areas etc;
- 3. The **minimum size of FGM** unit depends on tree species and FGM objectives (i.e. a minimum of 50 reproducing trees; in special cases, such as monitoring of the recovery of an endangered population, a lower number of reproducing trees is acceptable);
- 4. At least one tree species should be recognized as **target tree species** for each unit (in our case this is species for genetic monitoring);

EUFGIS minimum requirements are available at:

http://portal.eufgis.org/fileadmin/templates/eufgis.org/documents/EUFGIS_Minimum_r equirements.pdf Contents lists available at SciVerse ScienceDirect
Biological Conservation
LSEVIER journal homepage: www.elsevier.com/locate/biocon

Review

Translating conservation genetics into management: Pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity

Jarkko Koskela^{a,}, François Lefèvre^b, Silvio Schueler^c, Hojka Kraigher^d, Ditte C. Olrik^e, Jason Hubert^f, Roman Longauer⁸, Michele Bozzano^a, Leena Yrjänä^h, Paraskevi Alizoti¹, Peter Rotach¹, Lorenzo Vietto^k, Sándor Bordács¹, Tor Myking^m, Thröstur Eysteinssonⁿ, Oudara Souvannavong^o, Bruno Fady^b, Bart De Cuyper^p, Berthold Heinze^c, Georg von Wühlisch^q, Alexis Ducousso^r, Bjerne Ditlevsen^e ²an-European minimum requirements for genetic conservation units of forest trees.

Requirement group	Detailed requirements
Basic requirements	The unit has
	(1) a designated status as a genetic conservation area of forest trees, recognized by the appropriate authorities or agencies in a country
	(2) a management plan in which genetic conservation of forest trees is recognized as a major management goal. One or more tree species have been recognized as target tree species for genetic conservation in the management plan
	One of the following conservation objectives has been clearly stated for each target tree species within a unit:
	 to maintain genetic diversity in large tree populations; to conserve specific adaptive or other traits in marginal or scattered tree nonulations; or
	(3) to conserve rare or endangered tree species with populations consisting of a small number of remaining individuals
Population size	The minimum population size depends on the conservation objective as follows:
	Case 1: If the purpose of the unit is to maintain genetic diversity of widely occurring and stand-forming conifers or broadleaved species, the unit must consists of 500 or more reproducing trees
	Case 2: If the unit was established to conserve specific adaptive or other traits in marginal or scattered tree populations, the unit must harbour a
	minimum of 50 reproducing trees or, in the case of dioecious tree species with sexual dimorphism, 50 seed bearing trees.
	case 5. If the difficult of the service remaining populations of fare of endangered tree species, it must harbour a minimum of 15 unrelated reproducing trees.
Management	Silvicultural interventions are allowed within the unit and they are actively applied, as needed, to:
	 ensure the continued existence of target tree populations; and resta formulable conditions for growth and utility of the target tree conting and their natural comparation.
Monitoring	(2) create ravourable conditions for growth and vitality of the target tree species and their natural regeneration Field inventories are carried out every five or ten years to assess regeneration success and the nonulation size and to undate the management
inclusting	plan
	Between the inventories, the units are visited regularly to observe that they still serve their purpose and that they have not been damaged or destroyed
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ANE	
ELIECIC	
EUFGIS	European Information System on Forest Genetic Resources
	DATA MAPS SEARCH UPLOAD
Genetic conservation units	Home > Maps
Data standards	Units on Google Maps
Data providers	Chisters of units are indicated as red circles. Numbers inside the circles show how many units each cluster includes. You can zoom in to view the clustered
EUFGIS project	units separately.
Tools	Clustered markers 🗆 Show all
Links	Home view Selected view
Send feedback	Map Satellite
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FGM plot selection (3)

- 5. Presence of stand-level attributes paramount for FGM, representing the ecological adaptation of the population to the site (reproducing adult trees, presence and survival of natural regeneration (if expected based on the forest stand age), sexual and / or vegetative reproduction).
- 6. Availability of genetic data in the same or a nearby stand.
- 7. Avoidance of steep slopes or other topographical characteristics which might influence gene flow within the plot. (This criterion is not applicable for populations at the upper timberline or other special cases where steep inclination of terrain is unavoidable.)
- 8. All legal, administrative and silvicultural changes need to be documented.





FGM plot selection (4)

9. Priority should be given to plots for which stand history (e.g.: origin of genetic resources, year since last clear-cutting, timing of thinning operations, etc.) and high data density, especially in time series and precise plot documentation, is already available. For example, from the Gene Conservation Units (GCUs), experimental plots, yield and growth permanent observation plots, approved seed objects, national forest inventory plots, ICP Forest plots, etc. Background data is important to take into account during selection of monitoring plots and interpretation of monitoring results. Such background data includes: Climatic / environmental data; Soil data; Vegetation data; Data on past fructification and presence of natural regeneration, etc.;

10.Distance of the institution to the monitoring plot.

- 11.Easy accessibility of the plot (e.g.: road, footpath, rock obstacles, etc.). Whenever possible, select FGM plots that can be reasonably easily accessed, as this will reduce the workload in the field and the overall cost of FGM.
- 12.FGM plots can be promoted as 'research focal points' and included in other monitoring programmes and research projects (national inventories, ICP Forest, greenhouse gas emission/sink monitoring plots, forest soil biodiversity research and monitoring, etc. Such an approach would facilitate long-term continuation of monitoring activities at FGM plots, contribute to securing the long-term budgetary support and increase the amount of different types of data available for FGM plots).

FGM plot establishment

- When a site for FGM (i.e. a forest stand) is confirmed, a smaller area for FGM plot installation is to be selected within it.
- For stand-forming species the location for the FGM plot installation is chosen at random, while for scattered species a preliminary field survey of the selected stand for FGM is required.



Number of trees per plot (1)

- A minimum of fifty (50) reproducing trees for FGM.
- In rare cases, for scattered tree species only, the number may be reduced to 30 adult trees.
- An FGM plot for monoecious species consists of 50 unrelated reproducing trees and a minimum distance of 30 m between any two trees.
- For dioecious or functionally dioecious species 25 female and 25 male adult reproducing trees need to be selected with the same minimum distance requirement as for the monoecious species.





Number of trees per plot (2)

- If a tree is flowering, it is regarded as a reproducing tree. Therefore, the best time for FGM plot establishment and tree selection is spring, when potential trees are flowering; e.g. flowering cherry trees can be seen from far away.
- DBH and social class can be used as a proxy to identify a reproducing tree for monoecious species.
- For dioecious or functionally dioecious species trees must be selected during the flowering period to be able to positively identify the sex of trees.
- For species where clones or hybrids between target autochthonous species and allochthonous species occur, the selected trees first need to be genotyped for clonality or hybridisation.



FGM plot design. Stand-forming tree species

- When a forest stand is confirmed for FGM implementation, a centre of the FGM plot is to be randomly selected.
- Random sampling, as a way of sampling design, should be used as it is the only statistically safe option.



GPS coordinates of the centre;

Other trees are selected in concentric circles around the previously selected central tree with an increasing radius of 30 m by using a random azimuth observed from the central tree, important to ensure a minimum distance above but as close to 30m between any two trees as possible: (Bajc et al. 2020)

FGM plot design. Scattered tree species (1)

- Differences in spatial distribution and density among scattered species' populations, there is no universal approach for FGM plot establishment.
- Some species are present individually others occur in groups of different sizes in mixed forest stands or in specific patterns, the procedure for plot establishment is to be devised on a case-by-case basis.
- Common requirements of 50 unrelated reproducing trees and a minimum distance of 30 m between selected trees.





FGM plot design. Scattered tree species (2)

- In special cases of very low population density (e.g. endangered populations, edge populations), the number of trees can be reduced to 30.
- When a site for FGM implementation is confirmed, the locations must be additionally surveyed in more detail in the field.
- GPS coordinates of all suitable trees and NR sites / GIS software check / selection in the office.
- If the target species population is clearly visible and distinguished from other species in an orthophoto of the area, visual inspection of these photos may be used instead of the additional surveying in the field (e.g. *Prunus avium*).



FGM plot design. Scattered tree species (3). Populations in the form of clusters of trees

Individuals in clusters

- Several plots, which together form one FGM plot, should be installed in the field with the total sum of 50 trees.
- Clusters of trees must be located within the same stand, where the environmental conditions and the species composition are similar.
- The locations of tree clusters are plotted on the map in the form of polygons, which all together represent a sampling frame.
- Trees within a cluster should be selected randomly with minimum distance of 30-35 m inside each polygon.
- If the population density is not sufficient to carry out the process to find additional clusters.



Photo: Gregor Božič (SFI)



FGM plot design. Scattered tree species (4). Sporadically occurring trees

Sporadically occurring trees ("seek and find approach")

- In cases where the population occurs in small groups of only a few trees each or trees are present individually, random sampling with the requirements of a minimum number of reproducing trees (30-50) and a minimum distance of 30 m may become increasingly difficult.
- The area for tree selection could become too large and thus unmanageable.
- An initial surveying and saving tree locations may be too time consuming and labour intensive, especially where the terrain is difficult.
- It is advisable to get assistance from local foresters who are aware of the area and know where the target species is more likely to occur.
- All reproducing trees that meet the minimum distance requirement must be selected. If it is impossible to find 50 reproducing trees, all suitable trees should be selected, but not fewer than 30 trees (exceptionally in the case of endangered or edge populations!) with a minimum distance of 30 m from each other.

Natural regeneration (1)

- Inside an established FGM plot a greater number of NR subplots, if possible 20, should be established.
- NR subplots are to be used for several purposes: DNA sampling and NR abundance / mortality assessment.
- The establishment of NR subplots should be carried out after germination following each strong or massive fructification event, when fructification occurs every three to 12 years.



Table 3.2: Timeline of NR subplot establishment. Twenty new NR plots are established after each assessed fructification event. Preferably two fructification events are assessed per decade.

Year of monitoring	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Fructification event		•					•							•					•				
NR subplot establishment			٠					•							•					٠			

Natural regeneration (2)

- If fructification occurs every year or every second year, NR plots are to be established after a strong/massive fructification with approximately five years in between the previous and next round of NR abundance plots.
- Seed dormancy must be taken into consideration when planning the NR subplot establishment.
- For example, seed of Common ash (*Fraxinus excelsior*) usually remains dormant for two winters, meaning that germination and establishment of NR subplots will take place two years after the fructification event.

Natural regeneration (3)

- Natural regeneration centres should be surveyed in the field and their locations logged (GPS coordinates, the number of the tree which is the closest to the NR centre).
- From all regeneration centres, 20 of them should be chosen randomly for NR plot installation. If 20 or fewer NR centres are present, all should be used.
- Logging additional information about the location of NR plots, such as the distance (number of steps) and azimuth from the nearest labelled adult tree, is recommended to aid in finding these NR plots in the future.
- Inside each selected natural regeneration centre a 1 m² plot is to be installed and marked with metal rods.

COLLECTING SAMPLES

- Adult trees All labelled trees must be sampled (50 adult reproducing trees for monoecious species; 25 female and 25 male adult reproducing trees for dioecious and functionally dioecious species)
- For hybridising species and species with vegetative reproduction (clones), the initial number of adult trees to be sampled and genotyped can be higher (e.g. 100)
- Sampling of natural regeneration should be done at the 20 NR subplots (next to the abundance/survival 1m² plot) the third year after germination (3-year old plants).
- 50 NR samples are collected: 3 plants from 10 randomly chosen NR subplots, 2 from the other 10 subplots.
 For hybridising species and species with vegetative reproduction (clones), 100 saplings should be sampled and genotyped.
- Seeds are needed for DNA analysis and for seed testing at the advanced level of monitoring.
- Seeds must be collected from the 20 selected seed trees by climbing onto the trees and at least 200-300 seeds per mother tree must be sampled from several different branches (higher number of seeds are needed to be sure to have sufficient number of full seed), 20 full seeds per tree will be used for DNA analysis, for a total of 400 seeds.

Labelling of trees

- Each selected tree must be marked with a corresponding number and a band painted around the trunk to aid the visibility of the trees from all directions.
- Mark the central tree (number 1) with two or more bands to differentiate it from other trees.
- It is recommended to paint the number on the side of the tree that is pointing away from the central tree, as this helps locating the central tree, particularly from the outer rings of the plot.
- In some cases, it helps to label the trees on the side pointing away from paths or roads to avoid confusion with people seeking recreation in the forest.

Georeferencing

Selected trees within the FGM plot need to be georeferenced, which can be done at the same time as plot establishment.

Two georeferencing approaches are described in FGM manua:

1. The simplest way to georeference trees is to record the GPS locations of the selected trees using a GPS receiver.

However, this method is not suitable in the case of insufficient accuracy and/or precision of the GPS receiver. The accuracy of non-differential GPS receivers that are generally used by foresters can be as low as 15 m or more in mature forests (Simwanda *et al.* 2011). Differential GPS devices offer significantly better accuracy and precision (Zhang *et al.* 2014).

2. Another way of georeferencing trees is with the measured distance and azimuth from the reference point. The georeferencing calculator must be used to calculate tree locations.

PLOT DESCRIPTION (STANDARDISED FORMS DEVELOPED) (1)

- After establishment, the FGM plot should be described in detail in the "FGM Plot description" form, which is part of this Manual.
- All collected data is then deposited in a database.
- The form consists of two main parts: (i) plot description data and (ii) stand quality and description.
- Plot description data contains sections about the exact location, ownership, species composition of the forest stand, characteristics of the region, soil and climate. The silvicultural system, forest management objectives and designated status are also defined.

PLOT DESCRIPTION (STANDARDISED FORMS DEVELOPED) (2)

- The stand quality and description part is organised in such a way that one of the possible answers is selected for each descriptor.
- This part of the form describes the following:
 - forest health condition,
 - whether the forest is managed or not,
 - forest reproductive material collections,
 - natural regeneration,
 - vertical and horizontal structure of the stand,
 - slope,
 - quality of trees, etc.

Fagus sylvatica FGM plot (Germany) (1)

FGM PLOT DESCRIPTION

PLOT DESCRIPTION DATA

Monitoring tree species	Plot size		Stand age (from-to)				
Fagus sylvatica	Stands FGM pl	s area, ha 7, <u>1</u> ot area, ha 4,0	~150 year				
Exact positions*							
Latitude (I	N) Longitude	(E)	Altitude (m)				
48° 24' 25,00" I	N 11°	39'18,00" E	508 m.				
Ownership		BaySF					
Information about owner (<u>restricted</u> the parcel numbers, <u>Cadastrial</u> numl	information: ber, etc.)	BaySF Public fore Registration num	<u>st.</u> ber 091 810 241 332				
State Forest Office		Forstbetrieb Fre	ising				
Forest district		64. Kranzberger	Forst				
Forest compartment		7. Rehbuckel					
Forest sub-compartment							
	Latin specie	s name	Proportion, %				
Monitoring tree species and its proportion in the stands	Fagus sylvat	Fagus sylvatica ~66%					
Other species and its proportion in the stands	Quercus rob	ur	~34%				

Regional classification into growth areas (growth districts)	42 (<u>Tertiäres Hügelland</u>)
Bedrock	Obere Süßwassermolasse (eng. Upper Marine Molasse)
Phytocoenological association (according to Braun- <u>Blanquet</u>)	Natural forest association: <u>Luzulo</u> beech forest
Soil type (according FAO, 1971- 1981)**	Bodenart: Schluffe (Ausgangssubstrat Löss und Lösslehm)
Soil humidity (dry/mesic/wet)	Mesic (Wasserhaushalt: frisch)
Nutrient supply (rich/medium/poor)	Medium
Regional forest site classification	12.8 - Oberbayerisches Tertiärhügelland
Climograph	
Mean annual temperature (°C)	8,3 °C
Mean temperature during vegetation period (°C)	13,5 °C
Mean temperature of the warmest month (July) (C°)	17,3
Mean precipitation during vegetation period (mm)	520 mm
Ellenberg's climate quotient (EQ)	
Forest management objectives (Wood production-focused management; Habitat-focused management; Recreation/Aesthetics-focused management; Other type of management; Other type of	Multiple use focused management

Fagus sylvatica FGM plot (Germany) (2)

spaced

s

х

Date

Slope exposition

NE Е

SW

w

with

openings

with openings

х

SE

NW

Silvicultural system (shelter wood, coppice, continuous cover forestry, selective logging, etc.)	Positive selection fellings (Altdurchforstung)
Protected area (without use) yes/no	No.
Designated status (e.g. forest reserve, gene conservation area, protected area, etc.)	0,25 ha out of 4,0 ha is ICP Level II area.
If yes, since when taken out of management	

* Exact position is restricted information only for LIFEGENMON project use;

** FAO/UNESCO (1971-1981) The FAO-UNESCO Soil Map of the World.

STAND QUALITY AND DESCRIPTION

Forest health condition	Forest management	Stands history (origin)
Describe causes of poor or medium health below in remarks.		If planted - provide the origin of the FRM (i known).
good medium poor	Yes No	Naturally regenerated Plante
x	Managed X	x
Source	Natural regeneration	Structure of natural regeneration
Autochth Not- autochtho Unkno	Rare Modest Frequent	In Evenly distributed
onous nous wn	x	groups within FGM plot
x		x

Isolation	Fragmentation	Vertical structure of stands
Isolated from the nearest stand of the same species (400 m)	Species scattered within FGM plot (some grouping is visible)	
Yes No	Yes No	Single Two storied Multiple layered
Isolated X	Fragmented X	x
Horizontal structure of stands	Distance between trees / groups of trees	Genetic data
Openness and spacing of canopy		
Evenly Unevenly Evenly spaced spaced		

	Yes	No
	Availability X	
ilope	Quality of tree stems in general	۱

good	medium	poor
	х	

nds

Crown forms			Accessibility					
			Accessibility for seed collection / climbing					
good medium	poor		good	medium	poor			
x				x				

5-15%

х

15-40%

<0-5%

Past recordings of flowering, fructification (mast years), and seed collection

Past recordings	Year	Amount of collected seeds
Flowering		
Fructification		
Seed collection		

Remarks

Name / Surname / Signature

PLOT MAINTENANCE (1)

General maintenance

- Tree markings and NR plot markings must be checked periodically (every two years) and renovated if needed.
- Metal rods, used for NR plots marking, must be removed when monitoring of NR abundance is finished.

Replacement of trees

- If a monitored tree dies or is cut due to management, it must be replaced. The nearest suitable tree to the dead one should be chosen considering that the distance requirement of 30 m to the nearest monitored tree is fulfilled. Otherwise a tree from the periphery (preferably in the outer circle in the case of stand-forming species) of the FGM plot is to be selected.
- If the crown is damaged due to, for example, wind break, ice or snow break, but continues to fructify, the tree is kept for the monitoring. If the damage is too severe and fructification is not expected anymore, the monitored tree must be replaced.

PLOT MAINTENANCE (2)

- In species where more than 50 individuals are selected initially due to determination of clonality or hybridisation (by genotyping), any suitable individuals from these surplus trees can be used as replacements for lost trees. If clones were detected in the initial larger number of trees, the same genotype can be used as a replacement for the lost individual.
- The cause of the loss of a tree on the FGM plot must be determined and logged in the forms and in the database.
- Replacement trees have to be marked the same way as the original trees, but with consecutive numbers (51, 52, ...) to differentiate them from the replaced original trees (numbered 1 to 50).

Long-term maintenance of the plot

- Gaps in forest cover may occur even in close-to-nature forest management systems.
- In the case of the removal of a larger number of reproducing trees on the FGM plot due to forest management (e.g. irregular shelter wood), the plot should still be maintained and observations carried out with regard to NR abundance, flowering, and fructification. In such cases, the number of the remaining trees must be recorded at each observation.
- Such a situation, when FGM is severely limited due to a reduced number of reproducing trees may last for several decades, until enough younger trees reach reproductive age and meet the minimum requirements to be included in the FGM.
- The selection and replacement process should be undertaken over a longer period, so that the selected replacement trees are not biased towards the fastest growing individuals.

COLLECTION OF METEOROLOGICAL DATA

- Today CC is probably the main direct threat to genetic diversity and forest ecosystems. Indirectly it also enhances threats from diseases, pathogens, insects, fire and extreme weather events.
- Environmental factors play an important role in the reproductive success, growth and survivability of trees.
- In FGM many verifiers can be partially explained with changing environmental parameters, e.g. temperature and precipitation.
- To explain changes in various verifiers, it is therefore recommended to install meteorological loggers directly on the FGM plot.
- Data loggers and different environmental sensors. Meteorological loggers are cheap, easy to install and enable easy remote data collection.
- Meteorological data can also be obtained and extrapolated from nearby weather stations. This approach is however not recommended in locations with very heterogeneous conditions or microclimates.

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