



# Guidelines for conducting genetic monitoring in the field: European black poplar (*Populus nigra* L.)

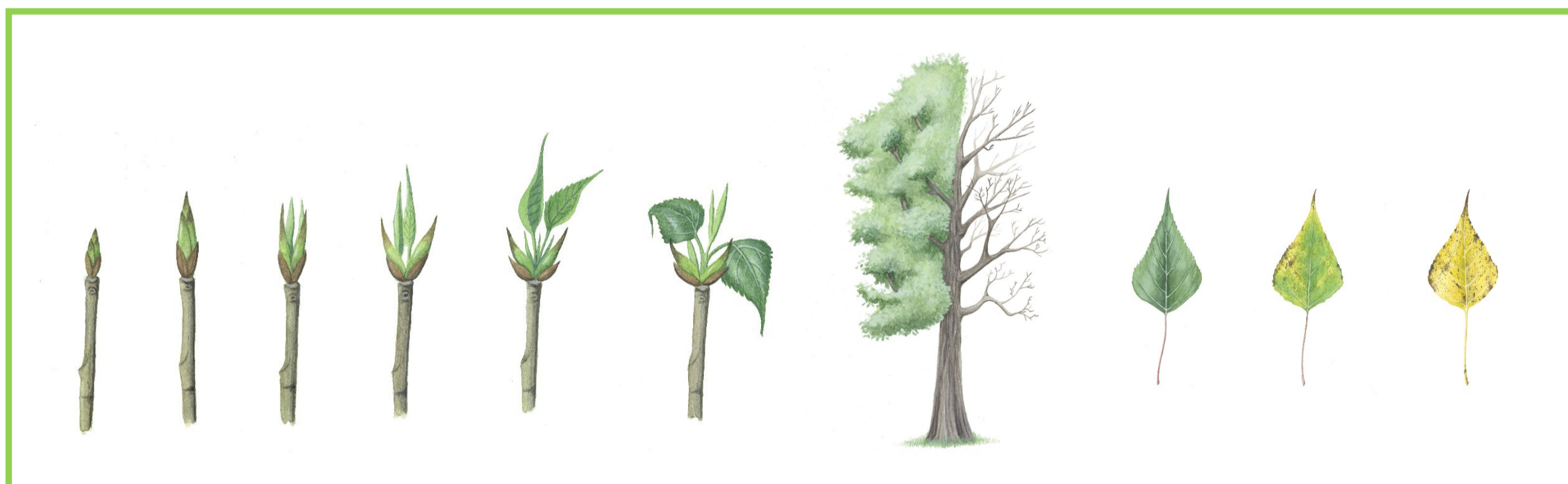
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*Populus nigra* illustrations made by Marina Gabor

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## INTRODUCTION

European black poplar is an ecologically important, dioecious and anemophilous, fast growing and short-lived pioneer forest tree species of mixed riparian forests with physiological adaptation to colonize open areas after disturbances and survive in changes associated with its dynamic river system. It enables natural control of flooding, is a keystone species for dynamic conservation and habitat restoration of floodplain forests and is considered as an indicator species for health and biodiversity of riparian ecosystems (Lefevre et al 2001, Rotach 2004). It naturally forms metapopulation of inter-linked local populations rather than isolated populations (Heinze and Lefevre 2001). The guidelines shortly describe the European black poplar, its reproduction, niche requirements and threats. They provide guidance on establishing and maintain a genetic monitoring plot and on recording all field level verifiers at the basic, standard, and advanced monitoring levels. To ensure representative sampling across the metapopulation is important to design a genetic monitoring system with randomly selected monitoring plots of adult reproducing trees in local populations and their natural regeneration centers along the river system. Genetic identification of *Populus nigra* (L.) trees must be performed by use of species diagnostic DNA markers in all monitoring levels. The main obstacle of forest genetic monitoring in the case of European black poplar is to find habitats where the mating system can develop in a natural way, and offspring can find regular, suitable mid- to long-term conditions.



## PLOT SELECTION AND ESTABLISHMENT

### FGM plot selection follows the metapopulation concept

➤ **FGM plot selection:** in riparian forests along the river system where interlinked local populations of *Populus nigra* (L.) forming a metapopulation structure. As metapopulation is mainly in exposure to high hybridization pressure due to introgressive hybridization from *Populus deltoides* & *P. x euramericana* clones from production plantations established in alluvial sites, it is necessary to confirm "pure" taxonomic status of selected *Populus nigra* (L.) trees also by genetic test with use of diagnostic DNA marker(s).

➤ **Field survey** – also in early spring, when the trees are flowering to ensure equal male and female sex ratio between selected trees.

### FGM plot establishment and maintenance

➤ **FGM plot design** – FGM area with 50 reproducing and taxonomical "pure" *Populus nigra* (L.) trees of different genotypes should be selected in local populations along the river system in sex ratio 1:1 of male and female. The monitoring plot in each local population should include at least 20 adult trees in maximum distance of 5 km.

➤ **Adult tree marking** – all 50 selected trees must be labelled with continuous numbers.

➤ Sampling design of **natural regeneration (NR)** follows the metapopulation concept of multiple regeneration centers (subplots) to capture the whole genetic diversity of *Populus nigra* (L.) and assess the risk of gene introgression and hybridization from exotic poplar species, and Lombardy poplar sources in the given area. The possible subplots of natural regenerations should be frequently monitored from April to June and where are occurring the newly seedling should be sampled immediately.

➤ Genetic test on NR-seedlings should be performed by diagnostic marker(s) for selection and further genetic analysis for "pure" *Populus nigra* (L.) samples.

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List of verifiers and background information with short description and observation frequency to be recorded during field work at the European black poplar genetic monitoring plots

	Name	Basic level	Standard level	Advanced level
Verifiers	Mortality / survival	Counting of remaining marked trees every 10 years and after every extreme weather event/disturbance	Same as basic level.	Same as basic level.
	Flowering	Individual tree level observation, during two major flowering events per decade, ideally equally spaced *	Same as basic level.	Same as basic and standard level, but flowering stage also recorded*.
	Fructification	Individual tree level observation, the same year as the assessment of the flowering at the standard level (regardless of the fructification intensity) *	Same as basic level.	Same as basic level.
Verifiers	Natural regeneration abundance	Expert opinion on the stand level on the newly germinated NR microsites (this observation is done also at standard and advanced levels).  Alternative: When no germinated NR were observed, then the NR centers of current year seedling should be estimated as alternative.	Observations on up to 20 NR subplots (NR microsites/centers) of only the newly germinated NR by sampling the seedlings app. every 5 years (after two major fructification events in a decade). No plants abundance counting in sampled area.	The same as standard level, or standard with increased frequency of observations – every year or after every major fructification event.
	Genetic test on NR-seedlings by diagnostic marker(s)	/	Testing a total of 100 seedlings on NR subplots, app. every 5 years (two fructification events per decade); if necessary, more of them are sampled to reach the required 50 pure-species individuals. In case new NR sites were not present under monitoring areas, then successfully established NR should be sampled and tested once per decade.	Same as standard level.
Background information	DBH class distribution	/	Measurement every 10 years.	Same as standard level.
	Height class distribution	/	Measurement every 10 years.	Same as standard level.
	Bud break	/	Individual tree level observation according to protocol, every 5 years.	Individual tree level observation according to protocol, every year.
	Senescence	/	Individual tree level observation according to protocol, every 5 years.	Individual tree level observation according to protocol, every year.
	Flowering synchronization	/	/	Individual tree level observation, during each assessed major flowering event.

\* At least one major fructification event should be assessed per decade. Basic level observations are used to identify major flowering and fructification events.