*SHORT COMMUNICATION*

# ORGANIZATION OF A MICROSATELLITE CONSORTIUM FOR *FRAXINUS* SPECIES

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*Fraxinus excelsior* L., the common ash, and *Fraxinus angustifolia* V., the narrow-leaved ash are noble hardwoods and the commercially most important species in Europe. *F. excelsior* is used in many European countries as a source of high value timber. Recently these two *Fraxinus* species have been recognized as important for long term conservation strategies (TUROK *et al.* 1999) in Europe in the frame of the Noble Hardwood Network of the European Forest Genetic Resources Program (EUFORGEN) set up by the International Plant Genetic Resources Institute (IPGRI/FAO).

A gene conservation strategy for European *Fraxinus* species has been proposed in the frame of this network (PLIURA 1999). Until now genetics of *F. excelsior* have retained little interest compared to oaks which is another important group of forest tree species in Europe. Despite its commercial use, the natural coverage of *F. excelsior*, which spreads through all over Europe and even extends into Asia, is known to have constantly shrunk in the past centuries, mainly because of deforestation for agricultural needs. Among other parameters, such a genetic conservation strategy would require information on the natural range of species, pattern of distribution, population structure, pollen and seed dissemination data.

Such requirements could be best fulfilled with the use of molecular markers, which seem to be the tool of choice for such programs (for a review, see LEFORT *et al.* 1999a). We recently made available the first nuclear microsatellite markers for *F. excelsior* (BRACHET *et al.*,1999; LEFORT *et al.* 1999b) and tested their conservation in *Fraxinus* and Oleaceae species. They proved to be usable in 11 other *Fraxinus* species including *F. angustifolia* and 12 other Oleaceae (LEFORT *et al.* 1999b). One microsatellite marker was specific of *F. excelsior* and another one was specific of *F. excelsior* and *F. angustifolia*. Microsatellite markers tend to be the favorite markers of gene conservation studies since they display numerous desired properties. They are usually highly polymorphic, they are co-dominant and thus enable distinction between homozygotes and heterozygotes. As they are DNA markers, they can be amplified from all kind of biological material from which a tiny amount of DNA can be obtained by micro-methods of DNA extraction and thus are not dependent of environmental or developmental factors. Microsatellites are widely regarded as being selectively neutral.

They have shown to be very powerful tools for genetic structure studies (STREIFF *et al.* 1998), pollen flow monitoring as well as hybridisation experiments in breeding programmes (N. FRASCARIA unpublished results). Microsatellites regions have also been identified and characterized in the chloroplast genome of *F. excelsior*. Using universal primers (WEISING & GARDNER 1999), 7 chloroplast microsatellites were amplified. Sequence data confirmed the presence of the mononucleotide stretches in the amplified fragments. A relatively high level of polymorphism have been detected: in particular high degree of genetic differentiation among populations was observed, as expected for maternally inherited markers (G. G. VENDRAMIN, pers. com.)

Because of the new declared interest in *Fraxinus excelsior* from the Noble Harwoods Network, we propose the organization of a *Fraxinus* microsatellite consortium which will gather all forest scientists interested in these *Fraxinus* species. This consortium will be managed by the company Agrogene, in the same form as it manages microsatellite consortia for major crops.

A microsatellite-enriched library has been built according the protocol of EDWARDS (1996) and is

available for dissemination. Preliminary works showed the library to be of a very high quality and very promising for further characterization.

Eighteen recombinant plasmid clones carried 21 distinct DNA sequences of *F. excelsior*, among which 23 microsatellite loci were detected. Twelve loci were characterized and 10 primer sets were published (LEFORT *et al.* 1999b). Provided enough laboratories are interested in investing in the further characterization of new microsatellite loci for *Fraxinus* species, such a common project could result in the short term in the availability of hundred of new microsatellite loci for *Fraxinus* species, triggering the development of *Fraxinus* genetics and possibly resulting in the elaboration of a genetic map for *F. excelsior*. Such a characterization project could also result in identifying alleles that could be region specific or species specific, as well as providing markers for other *Fraxinus* and Oleaceae species for which such a project would be too expensive.

In summary Agrogene will manage the maintenance of the *F. excelsior* microsatellite-enriched library, distribute the bacterial clones carrying the recombinant plasmids to the parties. The parties will sequence their inserts. All sequences will be collected by Agrogene, which will analyze them for providing in return with optimal amplifying primers sequences and annealing temperatures. Each party will retain intellectual property rights for its DNA sequences and primer sequences.

The task of each party would be to sequence a certain number of clones (for example 48 clones) and to test them with a few *Fraxinus* DNAs of reference. In return each party fulfilling this obligation will get preferential access to the totality of the microsatellite loci sequences and amplifying primers obtained through this project and this for a duration to be determined (six years by example), after which time the information (sequences, etc.) will be disseminated without restriction.

The estimated participation cost to the project is about 2,200 US$ per team. We call for all interested parties to contact Agrogene for further information or Francois Lefort for technical information.

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