

Population genetics of *Fraxinus* spp. in France: achievements and perspectives

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ABSTRACT

The genetic improvement of *Fraxinus excelsior* in France faces many challenges because of the special geographical position and the large size of the country. Different from countries like the neighboring Belgium, Germany or Switzerland, France has very varied climates that are either influenced by the Atlantic Ocean, the Mediterranean and by the continental regimes to the east. These particular conditions have shaped the genetic structure of the common ash and the close related species (*F. angustifolia*), and the development of the French Ash improvement program has evolved accordingly to better understand this underlying complexity. Therefore, we can summarize the French efforts in complementary programs that have looked into the ash species at different ecological and evolutionary levels, that can be divided in: a) country level studies of genetic diversity; b) country level studies of hybridization; and c) local gene flow studies (intra and inter-specific).

A) *Genetic Diversity - Large scale.* For the *F. excelsior* populations, located in north-eastern France, levels of genetic variability within and among stands were estimated with five microsatellite loci for the seedling and adult stages. As expected for a forest tree, our results reveal high levels of intra-population diversity and a low genetic differentiation between stands. However, a general and significant heterozygote deficiency was found for seedlings and for the adult trees (Morand *et al.*, 2002).



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For *Fraxinus angustifolia* populations, we determined the genetic diversity of *F. angustifolia* from southern France in five populations situated west of the Pyrenees and east of the Saône River, all in the Mediterranean basin. Results from the same loci used in common ash, indicate that *F. angustifolia* bears less alleles but at the same time, similar levels of inbreeding as common ash.

B) *Hybridization - large scale.* We sampled five well known populations of *F. excelsior* in north and eastern France, six populations in the Mediterranean area east of the Pyrenees and west of the Rhône river of *F. angustifolia*, and 23 populations in two areas that contain putative hybrid populations: Loire valley and Saône valley. Multivariate Canonical Discriminant Analyses indicate that intermediate morphologies do exist between the two species for the Loire valley, but not for the Saône populations. Genetic population assignment using the Bayesian procedures indicated that populations from the Loire valley cluster close to those of the Mediterranean populations of *F. angustifolia* as indicated by the morphological analysis.

Our results suggest that geographical separation may have favored different adaptations in both groups that may have undergone secondary contact in areas of intermediate ecological conditions. As far as recommendations for forestry practices, it is not advisable to use provenances from the Loire valley, as they show intermediate morphologies typical of *F. angustifolia*. For the Saône populations on the other hand, the present analysis does not show evidence of morphological introgression that can be attributed to hybrid individuals (Fernandez-M. et al. in press). In addition, dormancy responses have been examined, indicating that physiological introgression exists among common ash and the narrow-leaved ash, a feature that can be used as a diagnostic tool if used along with molecular markers (Gerard et al. in press).

C) *Local scale-Common Ash.* In the study of spatial genetic structure, we found a high level of within population. We confirmed a previous result (Morand et al., 2002) that is striking for a forest tree: the existence of a high heterozygote deficiency in this population. We have concluded that assortative mating or a Wahlund effect may play



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an important role in producing the observed heterozygote deficiency. The use of spatial autocorrelation tools revealed the existence of a fine-scale genetic structure in the Dourdan population. This means that gene flow by pollen and/or by seeds is restricted in this stand. All these results seem to indicate a general trend towards a fine-scale genetic structure in common ash populations that could be explained by differences in pollen or seed dispersal abilities between common ash and other tree species like oaks.

Local scale: hybridization process. We studied natural hybridization between the two ash species *Fraxinus excelsior* L. (common ash) and *Fraxinus angustifolia* Vahl (narrow-leaved ash) already known from experimental crosses (Raquin et al. 2002). The two species showing very divergent flowering times, we assessed the role of floral phenology in restricting gene flow at a local scale. We detected isolation by time patterns. Reproductive events mainly occur between co-flowering trees, and pollen flow is asymmetric. Early flowering hybrids are widespread, and show a higher male and female reproductive success, producing more flowers and fruits and suffering slightly less floral gale attacks. Otherwise they show relatively high selfing rates, and they may have a higher fitness in this type of intermediate ecotone, possibly increasing their colonizing abilities (Gerard et al. submitted).

Perspectives: Bearing in mind the previous results, and the limitations of a neutral molecular basis approach (see pioneering work for diagnostic markers e.g. Brachet et al. 1999), the tight links between the ecological adaptation, the distribution of the species and inter-species gene flow (hybridization), the complete understanding and “domestication” of the ash populations may need more sophisticated approaches, as those provided by the ecological genomics, using lessons from better studied (and funded!) species like poplar.



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