# Scientific report for the short visit ESF Research Networking Programmes grant 6350, titled: Conservation Genomics of European tree species in the light of climate change

## 1. Purpose of the visit

This short visit aimed to build up and strengthen collaborations between the Royal Botanic Garden Edinburgh and ETH Zuerich with benefits for both institutes due to their long existing and renowned expertise in the fields of conservation and ecological genetics and genomics. During the visit the aim was to exchange knowledge gathered in both labs on conservation genomic techniques to investigate long-term conservation strategies for European plant species. Furthermore, the purpose was to work on and develop a proposal outline on European restoration genomics, to establish key questions and particularly discuss appropriate methodological approaches which will be integrated into a joint restoration genomics project. More specifically, we planned to discuss how best to study the benefits and risks of non-local gene introgressions into natural habitats in European landscapes and its potential negative impacts on the adaptive potential (e.g. climate change) and resilience (e.g. pathogens, diseases) of species. Particularly in restoration programmes the effects of introducing non-local genetic material on long-term species persistence under climate change scenarios are currently poorly explored and understood.

In the past we would have been limited by the number of markers, by their lack of divergence between populations and by the fact that they are neutral. With the advent of next generation sequencing however we have vast numbers of markers that can be scored, admixture analysis should be very powerful for estimating e.g. gene flow from other populations even if they are only little differentiated. In addition it should be possible to make comparisons of the behaviour of markers as they travel from the introduced population into first the seed, and then into establishing progeny trees. With the right methodological knowledge this gives us a chance of following selection, subsequent to introduction of a population from outside the local area. During the visit in Zurich we wanted to further discuss these ideas and to establish the extent and feasibility of such a project and to determine the best funding agency for proposal submission.

## 2. Description of the work carried out during the visit

During the first week of the trip we drafted a pre-proposal for the above mentioned project and discussed newest conservation genomics technologies with the GDC (Genetic Diversity

Centre) at ETH and talked to laboratory staff on challenges and benefits of NGS (Next Generation Sequencing) to make sure that we apply appropriate methods in the proposal. Furthermore, we worked in close collaboration with Prof Richard Ennos from the University of Edinburgh (UE), Dr Felix Gugerli from the WSL in Zuerich, and Prof Johannes Kollmann from the Technical University Munich (TUM) who we thought would be great co-investigators for a restoration genomics proposal. The second week was dedicated to starting to elaborate the pre-proposal into a full proposal with the benefits of in house expertise for any arising issues concerning the methodological approach. I also participated in lecturing, by leading part of a discussion topic within the course "Resilience of Ecological systems".

## 3. Description of the main results obtained

The visit to ETH resulted in a proposal outline which is titled: "Restoration Genomics of native floras: understanding adaptive potential and resilience of European flora in the face of global change". This outline was submitted during the first week as a pre-proposal to the ERA-CAPS call in March 2014 and was further developed in the second week and will either be submitted as a full proposal to the ERA-CAPS scheme (in case of pre-proposal acceptance) in July 2014 or submitted to the NERC new investigator scheme in July 2014.

*Main ideas that will be outlined into a full proposal*:

Humans have radically altered the natural landscape across Europe over the past 2000 years. The fragmentation, degradation and modification of forests, grasslands and other terrestrial ecosystems have had major consequences for the provisioning of ecosystem services, and will also affect biodiversity conservation and climate change mitigation. In recent decades there has been an urgent need to reverse these trends as indicated by international initiatives to restore natural ecosystems, through the Convention on Biological Diversity (see Aichi target 15 of CBD).

More recently, humans have also been responsible for the movement of a broad range of genetic material throughout Europe with increases in the open trade of plant material linked to landscaping and restoration. This anthropogenic dispersal of plants may have led to significant changes in the genetic and ecological structure of native flora. It is likely that the introgression of new genes into native habitats will influence species' local adaptation and resilience to their specific habitats. Particularly, in areas where large-scale planting of non-local material has taken place next to small native woodlands, gene flow between these populations is very likely. Until recently it was impossible to quantify the extent of such introgression of adaptive genes into native plant populations due to anthropogenic movement of germplasm. Previously, we were limited to using molecular markers with limited genome coverage unable to capture regions under selection. State of the art next generation sequencing (NGS) approaches offer a method for understanding these impacts. This includes the implications of proposed climate change adaption strategies such as 'predictive provenancing' where selected provenances are planted in novel sites based upon models of performance and projected climate change. Such strategies could massively increase the frequency of introgression of non-local genotypes into native populations with presently unknown consequences.

The objective of this project is to apply state of the art NGS technology to advance our understanding of how the adaptive landscape of ecologically and economically important plant species are altered by such unintentional anthropogenic gene flow. Recent advances in NGS, specifically through the establishment of large Single Nucleotide Polymorphism (SNP's) based genotyping, make the evaluation of gene flow among populations at the level of adaptively relevant genes feasible across multiple species and landscapes. By combining such an approach with screening plant species important in restoration of natural habitats (non-model organisms) we will significantly contribute to the science of restoration genomics. Specifically we will a) identify local adaptive genetic diversity important for resilience and persistence of European landscapes, b) evaluate the frequency of introgression of non-native genes into native populations, and c) understand the implications of anthropogenic introgression for native plant genetic resources.

*Methods:*

Long-term common garden and transplant experiments have demonstrated that local adaptation within species is common yet we have limited knowledge of the genes underlying this adaptation. In the suggested project we will combine genome-wide re-sequencing with *De-novo* assembly of reference genomes to examine adaptive variation in ecological and economically important species of temperate forest trees, shrubs and herbs. Specifically, we will sample reference populations of (I) historically isolated (pure native stock), and (II) introduced population (non-native stock), as well as (III) seeds and (IV) established progeny to explore how introgressed genes are filtered through early reproduction and recruitment. This sampling approach will be stratified across species ranges to account for climate variation using longitudinal and altitudinal gradients to maximize variation for drought and cold tolerance. This will enable us to explore the levels of adaptive variation for critical environmental variables likely to be under strong selection as well as to infer introgression of non-native genes.  Such traits are predicted to be especially relevant to climate change.

*Exploring adaptive variation:* DNA sampled from adults, seedlings (established juveniles) and seed will be pooled and sequenced with 80x coverage using Illumina. Because the cost of sequencing are rapidly declining we expect to be able to repeat this approach for at least 48 sites (8 populations and 6 species) with at least three comparative studies of species across different countries), although with the plummeting costs and rapidly increasing throughput of DNA sequencing we predict that several other species may be feasible included. Illumina reads will be mapped onto reference genome sequences, of related taxa (such as found in the Rosaceae, Fagaceae and Betulaceae). The population genetic analysis of the pooled sequence data will be analysed using the software PoPoolation (Kolfer et al 2011) and with support of Bioinformations from both the Edinburgh Genomics Centre and Zurich Genetic diversity and Functional Genomics Centres. Genes showing the greatest levels of polymorphisms (high degree of differentiation among sites) will be identified and subsequently analysed for Gene Ontology. Candidate polymorphisms including adaptive variation for soil type, cold tolerance and drought stress will be identified.

*Identifying gene introgression:* To evaluate the extent to which important adaptive variation is introgressed into native populations we will use *De Novo* SNP discovery using Double Digest RAD sequencing. This technique makes it feasible to evaluate sequences from large numbers of individuals. We will isolate SNP's from the same four sample groups (I-IV) for six species and using admixture analysis determine the proportion of a) pollen that is being introgressed from non-native to native stocks, and b) the proportion which are surviving in the established progeny. Subsequently these SNP's can be mapped against the candidate genes for specific adaptive traits. Ultimately, this will enable us to distinguish among markers which are favoured and those that are selected against.

The work plan for the project is envisaged for 3.5 years: Years 0 (own contribution pre-project start) and 1 will include an initial planning and coordination phase among partners, coordinated recruitment of relevant staff Postdoc/PhD students, planning and execution of sampling and mapping including DNA extraction for evaluation of adaptive variation and introgression of non-native genes. Year 2: Illumina sequencing, population level genetic analysis, *De-novo* SNP discovery, genotyping and admixture analysis. This will be conducted in parallel among the partner institutes. Year 3: Identification of candidate polymorphisms for adaptive variation, completion of molecular analysis, creation of database, write-up of papers, and Year 4 (own contribution post-project) wrap-up project consortium project meeting and planning of future work on restoration genomics.

*Significance of study:*

Maintaining the adaptive potential of plant genetic resources is an international priority. Outbreaks of diseases (Ash dieback, Elm disease, acute Oak decline) or the spread of detrimental invertebrates (Asian long-horned beetle, nematodes) hint towards negative effects of broad-scale transportations but also show the importance of a high genetic diversity for species to cope with these pests. Yet plant material is being transported across Europe and the implications of this homogenization of genetic diversity remain unknown. This project will provide the scientific knowledge to better inform management of plant genetic resources relevant to habitat restoration across Europe. Many restoration projects are sowing seeds and planting trees but rarely consider genetics and thus there is the urgency to develop appropriate guidelines. The project results will help ensure a) maintaining viable locally adapted populations, b) informing future management, c) developing mitigation strategies for past mismanagement, and d) reducing economic costs due to maladapted populations.

One important application of these results will be in the verification of the ideas surrounding predictive provenancing. There may be a number of unexpected consequences of future transfer of genetic material to adapt to climate change, but without empirical evaluation of how genes move among native and non-native populations and how this might undermine local adaptive variation we have little ability to predict the advantages of such future strategies.

The results from this project will advance our knowledge of how best to design seed sourcing for restoration and how best to manage genetic resources of keystone plant species in the face of global change. This will enable the refinement of guidelines for seed sourcing and recipient site matching for restoration with matching natural adaptation to site conditions. The combination of genomic, ecological and modelling approaches will unravel how to introduce a set of useful traits into forest habitats without disrupting important adaptive traits and genetic diversity, to inform policy and management. This information is vital to conserving the evolutionary processes which underpin ecosystem services provisioned by natural habitats and will help ensure the long-term success of restoration projects of great economic and societal value.

## 4. Future collaboration with host institution

In addition to strengthening the already existing collaboration between ETH/WSL and RBGE/UE this project also created new scientific links between the RBGE/UE, ETH/WSL and TUM. The collaboration between these institutes will enable us to have broad-scale European projects with local expertise to tackle novel plant genomic questions, e.g. in restoration and conservation projects. This collaboration is a fundamental prerequisite to maximize the utility of the limited resources available and to meet the European demand for molecular plant science innovation with a long-term perspective. Collaboration between RBGE and ETH particularly will continue in the future through joint projects and publications, knowledge exchange, staff exchange and visits.

## 5. Projected publications / articles resulting or to result from the grant

During the visit it wasn’t possible to generate data that would lead into a publication directly linked to the grant. Nevertheless, the grant helped collating state of the art, collaborative, transnational research in molecular plant genomic science into a high-quality research proposal which stands high chances of retrieving funding.