Reg. No: 2015/18/M/NZ2/00394; Principal Investigator: prof. dr hab. Robert Hasterok

The world is currently entering a period of climate change where plant populations will experience different environmental conditions. As a result, natural selective pressures will drive evolutionary changes that could result in some plant species changing in function and appearance or even becoming extinct. Equally, new species could appear that could displace pre-existing populations. These possibilities are the source of considerable concern as there will be resultant changes in what are called "ecosystem services". Thus, change in plant populations will alter soil fertility by changing interactions with nutrient cycling microbes. Difference in root architecture and growth pattern will impact on water movements (together with particulates and soluble nutrients such as nitrate) that could increase or decrease entry into river systems. All of these would also impact on animal populations from insect pollinators and pests to fish.

Measurements of any potential shift in the plant population therefore represent an important part of environmental monitoring to deal with the effects of environmental change. A large number of such studies are currently in progress but they are mostly linked to measuring changes in the DNA sequences in plant populations. However, this ignores a series of mechanisms through which plant form and function is controlled, which are cumulatively known as epigenetic mechanisms. These include direct modification of DNA sequences via methylation of cytosine nucleotides within the DNA as well as methylation and/or acetylation of protein histones around which the DNA molecule is wound. Such modifications influence the structure of the DNA-protein complexes that make up the chromosome and thereby have large-scale effects on patterns of gene activity. Importantly, epigenetic changes are imposed by enzymes whose activity is influenced by environmental conditions and these changes are most often not revealed through simple examination of DNA sequences; and so may be considered to be hidden from most studies. **Our project aims to describe variation in both DNA sequences (genetic) and epigenetic changes and correlate these with different selection pressures in a series of natural environments.** This innovative project will establish a model approach through which scientists will investigate if climate change is affecting variation amongst plant populations.

The study will exploit the grass species *Brachypodium distachyon* (hereafter Brachypodium) which the international team of applicants have played a major role in developing as an widely accepted model system. Focusing on Turkey as a centre of Brachypodium diversification new collections of this species will be collected from the six main climatic zones. Fifty plants will be collected from each climate zone representing a total of 300 new lines. This represents an important step as existing Brachypodium collections due to multiple cycles of propagation under conditions different from those where the plants were originally isolated and so they will have lost much of the original epigenetic information. Each plant in the new collection will be accurately described using the National Plant Phenomics Centre (NPPC) at Aberystwyth University, UK. The NPPC will use computer-assisted image analyses approaches to provide accurate descriptions of every plant form and also how this changes on the imposition of drought; a major limiting factor in determining the distribution of Brachypodium. These data will provide the basic descriptions of plant form and physiology to correlate with geographical origin and genetic and epigenetic variation.

To reveal both genetic and epigenetic variation, a cutting edge DNA sequencing approach known as Genotyping by Sequencing will be employed. This will derive DNA sequence information that will be used to identify very small changes known as single nucleotide polymorphisms variation in which are taken to reflect the different selection pressures. By changing the method that the DNA is derived it is possible to use the technique of bisulfite sequencing to reveal the different methylation patterns in each line. Genetic and epigenetic variation will need to be related to gene expression patterns and this will be determined. Therefore, the project will determine how far genetic/epigenetic variation correlates with environmental origins, plant form and function (phenomics) and gene activity (transcriptomics).

Importantly, these focused investigations will be complemented by larger scale chromosomal studies as epigenetic changes can result influence chromatin structure to such an extent that it can be detected at the microscopic level. The consortium encompasses world-leaders in chromosomal studies in Brachypodium so that changes will be defined; particularly in response to stress (here salt treatment as a well-established experimental system) and correlated with data derived in all parts of the programme.

This ambitious programme will be the first time that phenome, genome, epigenomes and chromosome structures with environmental conditions. It is proposed that this project will reveal the importance of monitoring the epigenome in determining if a plant population is experiencing altered natural selection pressures in response to climate change.