

**Plant evolution is a complex process** which can lead to many changes within chromosomes. Differences in genome size and chromosome number among closely related species may indicate complex evolutionary history. Old World lupins (OWLs) is a group characterized by great diversity in genome features among species. It is suggested that large scale genome changes (e.g. whole genome duplication) can be the reason behind such diversity, however the exact mechanism and structural changes which emerged from this processes are still unknown.

**The main objective of this project is to decipher the chromosomal restructuring which happened during lupins evolution. This survey will be performed on selected species from Old World lupins, which differ in evolutionary age, genome size and chromosome number.**

In this project we hypothesize that:

1. OWLs shares substantial genomic regions conserved among species. Some chromosomes undergone more complex reshuffling, while the others remained intact during evolution.
2. Rearrangements which happened during evolution can be inferred from cytomolecular tracking of blocks corresponding to particular chromosome regions.

Cytogenetics has been used to understand chromosome structure and genome evolution in both animals and plants. Fluorescence *in situ* hybridization (FISH) is a widely applied chromosome identification technique, facilitating tracking of chromosome rearrangements and comparative mapping. It was used successfully in karyotyping of model species: *Arabidopsis thaliana* or *Brachypodium distachyon*. In that case large BAC clones were used as molecular probes.

A similar approach was applied in our preliminary studies, involving the comparative mapping of *Lupinus angustifolius* (reference species for genus *Lupinus*) with four wild lupin species (*L. cryptanthus*, *L. cosentinii*, *L. micranthus* and *L. pilosus*). The obtained results were sufficient to infer a general overview of chromosomal rearrangements, but due to limitation in detection method (only regions covered by BAC clones were studied) the structural changes in whole chromosome were not known. In order to precisely track the evolutionary changes it is necessary to design a new type of probes, covering the length of entire chromosome. It is possible to combine thousands of short sequences (oligonucleotides) in one FISH probe. This technique, called 'oligopaints' is capable of hybridization to sequence of whole chromosome which effectively 'paints' it. It was already utilized for 'painting' of two cucumber (*Cucumis sativus*) chromosomes, and comparative mapping with other species of the genus *Cucumis*.

In the proposed project, we are going to generate a set of probes specific for arms of selected chromosomes. Each probes will consist of up to 20 000 oligonucleotides. These probes will be designed on template based on genomic sequences of selected *L. angustifolius* chromosomes, combined with sequences generated from next-generation sequencing (Miseq) for three wild lupins. We will also generate species-specific markers based on a simple sequence repeats of DNA (SSRs). SSRs with the pre-selected BAC clones, specific for selected chromosomes will be used in conjunction with oligonucleotide probes in FISH. Proposed combined approach, will allow to detect the exact location of the chromosome rearrangements among studied species and will expand our knowledge about their structural changes, and karyotype evolution.