In cell biology, various mechanisms are responsible for processes such as controlling cell division, the formation of gametes (like eggs and sperm), and fertilisation. These mechanisms aim to ensure the exact number of chromosomes was reproduced for a given species. Nevertheless, in plants these processes are often disturbed. Such disruptions can lead to the doubling of the usual set of chromosomes (which represent the basic "genome" leading to the formation of an "autopolyploid", containing more than two identical genomes in its cells). Alternatively, a polyploid can be formed by crossing related species followed by the spontaneous chromosome doubling of the resulting interspecific hybrid. Such a scenario increases the chances of successful gametes formation to make fertile offspring. These offspring are new species and are called "allopolyploids". It is well known that newly formed polyploids are genetically unstable due to the activation of "jumping genes" (mobile genetic elements), altered meiosis, chromosome rearrangements and changes in both the number and expression of their multiplied genes. Despite these facts, it is believed that most plant species underwent at least one polyploidisation event in their evolutionary history. It may be linked with the fact that polyploidy, allopolyploidy in particular, is associated with enormous genetic plasticity, which under certain environmental conditions can provide a selective advantage, allowing the occupation of new ecological niches. Allopolyploids are often found among economically important crops like wheat, oats, cotton, tobacco, oilseeds like rapeseed, and sugar cane. Therefore, numerous studies have aimed to better understanding their genetic (which includes changes at the DNA level) and epigenetic (which includes the modification of DNA by methylation of some nitrogenous bases and chemical modifications of histone proteins that form a complex with the DNA called chromatin) processes that drive their evolutionary success.

Unfortunately, studying allopolyploids, especially those belonging to the grass family, is often very difficult due to their large genomes with an extensive content of repetitive DNA sequences. *Brachypodium hybridum* will be used in the research project because it is the model organism for studying polyploidy in plants. Despite its allopolyploid nature, it has a small nuclear genome with fully sequenced genome. It is estimated that *B. hybridum* arose c. 0.5 - 1 million years ago by crossing two species resembling the modern *B. distachyon* and *B. stacei*. Recently, these two putative progenitors have been successfully crossed in the laboratory, leading to the formation of an "artificial" *B. hybridum* (a so-called resynthesised allopolyploid). It provides a unique opportunity to track changes at the genetic and epigenetic level involved in the stabilisation of the newly formed species.

One of the most important tasks in our project is to explain the mechanisms responsible for the spatial organisation of the nucleus in the allopolyploid species. The studies will be performed using methods that allow the microscopic visualisation of specific chromosomal domains (e.g. centromeric and telomeric) and entire parental genomes in nuclei. Moreover, the state-of-the-art CRISPR-dCas9 genome editing technique will be used to localise selected DNA sequences in the nuclei of living cells. It will also compare the distribution of centromeric and telomeric domains in the nuclei of the evolutionary "old" allopolyploid and its "young" resynthesised counterpart. Also, the roles of selected nuclear envelope proteins on the distribution of centromeres and telomeres in these allopolyploid nuclei will be verified. For this purpose, *B. hybridum* plants with some precisely mutated nuclear envelope proteins genes will be obtained using the CRISPR-Cas9 or CRISPR-Cpf1 genome editing methods.

The fate of duplicated genes is particularly important for understanding the evolution of polyploids. Part of the gene copies may undergo inactivation due to changes in the DNA sequence, while others can be reversibly switched-off and switched-on in an epigenetic way. A special kind of gene silencing in allopolyploids in which the ribosomal RNA (rRNA) genes derived from one of the progenitors are inactivated is called nucleolar dominance. This phenomenon has also been found in many lines of *B. hybridum*. Therefore, the main goal of another research task in this project is to determine the mechanisms underlying the nucleolar dominance in the studied allopolyploid and its resynthesised counterpart. Advanced methods in molecular biology, together with next generation sequencing techniques, will be used to find out if the rRNA genes derived from the genome of *B. stacei* have been irreversibly inactivated in a genetic manner, or their sequences have only been silenced in an epigenetic way.

This project should provide a better understanding of the key biological mechanisms underlying the evolutionary success of polyploids. Its results should be applicable to other, economically important allopolyploid crops.