Final report

Interspecific hybridisation is a process of interbreeding between individuals of different species. Plant species are more prone to hybridise than animal species. In certain groups of plants interspecific hybridisation can have evolutionary consequences. The hybrids are sterile but they can regain fertility by genome duplication. The allotetraploid hybrid lineage can becomes gradually isolated from the parental populations (hybrid speciation) or can backcross with one of the parents, producing a new hybrid in which the genome of this parent becomes will dominate. Repeated backcrosses lead to genomes in which the other parent is only represented by small mosaic or only individual genes (introgression). Over the past two decades, traces of interspecies hybridisation have also been detected in certain groups of fungi but „true" hybrids having complete genomes from two species were rarely found. In most cases, the genomes had chimerical structures composed of admixed mosaics of the genomes of the hybridising species. Although many chimeric yeast strains have been isolated mainly from industrial environment, astonishingly little is known about how these strains might have arisen. Our laboratory was among the first to investigate the phenomenon on lab-made „synthetic" hybrids in two ascomycetous genera, Saccharomyces and Metschnikowia, as models. The current OTKA project was based on our previous results published in numerous papers. The major new results achieved thanks to the OTKA grant can be summarised as follows.

1. The hybrids of plant species and animal species are sterile because they cannot form functional gametes. The failure of gamete production is mainly due to the inability of the allosyndetic (homeologous) chromosomes to pair during meiosis. This sterility barrier can be circumvented in certain groups of plants but duplication of the allodiploid hybrid genome. Our research carried out before this OTKA project revealed that genome duplication can also take place in interspecies yeast hybrids, but it does not restore fertility. We hypothesized that it might be attributed to the specific mating system of yeasts. In this study we experimentally verified the hypothesis (e.g. Sipiczki et al., 2020). In the genus Saccharomyces, used as model to study the consequences of genome duplication, the central regulator of sexual processes is the MAT locus. It contains the genes that switch on or off two alternative programs: mating and gametogenesis (meiosis and sporulation). The haploid cell can mate (fertilise) but cannot form gametes (ascospores). It has one of the two MAT cassettes in the MAT locus: either MATa or MATalpha. During vegetative propagation, the cassette in the MAT locus can be replaced with the other cassette stored in a silent locus (mating-type switching). When two haploid cells having different MAT cassettes mate, they form a heterozygous MATa/MATalpha zygote. The two cassettes interact. Their interaction switches off the mating programme and switches on gametogenesis. Thus, the diploid cells cannot mate but can form gametes (ascopores). The gametes of the allotetraploid plant hybrids are usually functional, thus genome duplication makes the interspecies plant hybrid fertile. We demonstrated in this project that the gametes of the yeast allotetraploids are not functional. Their sterility (inability to mate/fertilise) is due to their MATa/MATalpha heterozygosity that blocked the mating programmes and mating-type switching. For the analysis of the MAT genotypes in the hybrids we had to develop cassette-specific and species-specific primer pairs. Using these primers we could examine the MAT genotypes in two-species and three-species hybrids and their alloaneuploid segregants. In alloaneuploids that lost the MAT-carrying chromosome in one of the subgenomes and thus also MAT heterozygosity, both the mating programme and the mating-type switching mechanism became active. These segregants were fertile, but they were no longer hybrids since one of the subgenomes was incomplete (nullisomic for its MAT-carrying chromosome). The results are published in Sipiczki et al. 2020.

2. When investigating the duplicated (allotetraploid) genomes, we noticed that the allotetraploid cells lost chromosomes quite easily during propagation. The current project revealed that the chromosomes were not lost randomly. The loss of the first chromosome destabilised its subgenome. This subgenomes then lost additional chromosomes more frequently than the other subgenome. Thus, the originally hybrid genome gradually lost large parts of one of the subgenomes. Since recombination took place occasionally between the (allosyndetic) chromosomes of the subgenome, the gradual size reduction process lead to chimeric genomes composed of mosaics from both parental genomes

(frequently chimeric/recombined chromosomes). We performed these studies on lab-made synthetic hybrids but we hypothesize that this sort of postzygotic events have shaped the chimeric genomes found in yeast communities of traditional fermentation technologies. The results and observations are presented in Karanyicz et al., 2017 and Sipiczki, 2018.

3. To learn more about the consequences of the postzygotic evolution of the hybrid genome we chose a group of strains which we found in previous projects to have chimeric primary barcodes. The barcodes are short segments of evolutionary conserved genes used for taxonomic differentiation of species. We found that the type strains of the pulcherrima-clade species of Metschnikowia (8 species) had highly heterogeneous rDNA barcodes (the D1/D2 domains and the ITS segments of the rDNA repeats, the so-called primary barcodes). From network analyses of cloned barcode sequences we inferred that they had chimeric rDNA arrays. In this project we extended the investigation to the so-called secondary barcodes such as ACT1, EF2, TEF1, RPB2. We found that most strains were heterozygous for these genes, some contained more than two copies and the copies differed in sequence. In certain cases the intrastrain differences were bigger than the interstrain differences, indicating that these genomes harboured genes of different evolutionary histories. Prompted by these results we set out to analyse the genome sequences of four strains for homo/heterogeneity of a larger group of conserved genes. This analysis, as expected, corroborated the assumption (based on the barcode results) that chimeric genome structures occur in these "natural" strains. Then we tested the type strains for sexual compatibility. Some of them formed viable hybrids. Thus, genomes containing genes from different species can evolve from hybrids in these yeasts as well. The major findings of these experiments are described in a paper recently accepted for publication (Sipiczki, 2021).

4. A further interesting result of our research preceding this project was the observation that the mitochondrial genome was uniparentally inherited during hybridisation. Here we demonstrated that although the hybrids usually receive the mtDNA from one parent, recombination can occasionally take place (e.g. Szabo et al. 2020). We compared the PCR-RFLP patterns of the mitochondrial genomes of the parental strains, their hybrids and certain segregants. All hybrids were homoplasmic, we never detected two different mitochondrial genomes in the same hybrid. The hybrids usually received their mitochondrial genome from one parent. Hybrids having mtDNA from the other parent or having recombinant mtDNA were much less frequent. From these results we inferred that the hybrid nuclear genome is compatible with both types of mitochondrial genomes and also with their recombinants. We localised the recombination sites in certain hybrids by RFLP analysis but the sequencing of the entire mtDNAs by Oxford Nanoprobe technology is still underway. The mitochondrial genomes of the hybrids did not change during segregation. The major findings of these experiments were published in Szabo et al., 2020.

5. The new results debunk the rather widespread notion that nucleo-mitochondrial incompatibilities make the hybrids sterile. According to our results, such incompatibilities only cause respiration deficiency (not lethal in Saccharomyces and related genera!) and, moreover, do not do so in the hybrids but only in some of their segregants of chimeric nuclear genomes (Szabo et al., 2020). As long as the nuclear hybrid genome is complete, the hybrid is sterile regardless of the inherited mitochondrial genome. When the hybrid loses MAT heterozygosity by losing a MAT-carrying chromosome, the alloaneuploid descendant becomes fertile, regardless of its mitochondrial genome. However, when additional chromosomes are also lost, certain descendants become respiration deficient. As respiration proficiency is required for the sexual processes, these descendants cannot mate and cannot sporulate. These results are also described in Szabo et al., 2020. We hypothesize that these chromosomes contain genes necessary for the maintenance of the activity of respiration-specific mitochondrial functions. These genes may have different alleles in different species. If the allele compatible with the mitochondrion is lost by the los of the chromosome, the cell loses the ability to respire. Tests of this hypothesis are underway.

6. Other laboratories described strains isolated from natural substrates that had barcode sequences from three species. Prompted by these observations we asked whether three species can also be hybridised. In the paper Sipiczki, 2019, we described two strategies. (1) Hybridising a fertile chimeric

segregant of a two-species hybrid with a haploid strain of the third species. (2) Hybridising a sterile two-species hybrid with a haploid strain of the third species. Both strategies proved to be applicable but both have drawbacks. With the former method only chimeric three-species strains can be produced, because one partner is already chimeric. The product of this hybridisation has a complete genome of the third species and only mosaics from the other species. The latter strategy requires two heterothallic strains that have stable (non-switching) mating types. One has to be of a-mating type, the other has to be of alpha-mating type. Their hybrid is sterile and stable (low segregation rate). By definition, a sterile strain cannot be hybridised with other strains. But very rarely, in very few cells, their mating programme escape the MATa/MATalpha suppression and mate with a mating proficient haploid cell. We made use of this observation and managed to obtain hybrids of two species by "mass-mating" of large populations of the two-species hybrid and a third species. Preliminary results are described in Sipiczki et al., 2020. The FACS analysis determined triploid genome size in these hybrids and we detected all chromosomes of all parental strains in their genomes by pulse-field gel electrophoresis and PCR-RFLP of chromosome-specific marker genes. A publication reporting on these results is in preparation.

7. Based on the available literature data and our own results, we proposed a "unifying" model for the mechanism underlying the biological isolation of the species of the genus Saccharomyces. The model is described in Sipiczki, 2018. The currently valid taxonomic division of the genus is mainly based on the biological species concept: the species are reproductively isolated because their hybrids are sterile. We found that the reproductive isolation is ensured by a "double sterility barrier". The first and second barriers operate independently and are underlain by different mechanisms. The former barrier operates in allodiploids, the latter in allotetraploids, provided the allodiploid genome becomes duplicated. The allodiploids are sterile because the chromosomes of the subgenomes cannot pair at the beginning of meiosis. Without chromosome pairing no meiosis can take place and no viable ascospores (gemetes) can be produced. The second barrier takes effect when the allodiploid genome doubles itself. Upon genome duplication the first barrier no longer operates because the subgenomes are duplicated and meiotic chromosome pairing can take place within the subgenomes. In fact, the allotetraploid meiosis is autodiploidised. Thus, the allotetraploid can produce viable gametes (ascospores). Genome duplication is sufficient to make a plant hybrid fertile, but it does not make the yeast hybrid fertile because the gametes are heterozygous for the MAT cassettes and the MAT heterozygosity supresses the mating programme. Thus, the meiotic division of the allotetraploid does not restore fertility but returns the hybrid to the sterile allodiploid state. This mechanism is yeast specific and was not known before our work. However, neither barrier is absolute. Occasionally, asymmetric cell divisions can occur, leading to the formation of aneuploid daughter cells or ascospores. If Chromosome III is lost, the cell loses MAT heterozygosity because the MAT locus is on this chromosome. The loss of MAT heterozygosity makes the cell fertile, but at the same time also unstable. Additional chromosomes can be lost and the genome gradually becomes smaller and smaller. Since genome reduction occurs mainly in one of the subgenomes and recombination can occasionally take place between homeologous chromosomes, the process leads to chimeric genomes composed of a nearly entire genome of one species and mosaics of various sizes from the other species. The hybrid genome can be reduced in the course of vegetative propagation of the cells (we call this process GARMi) or at meiotic divisions (wee call this process GARMe). A detailed description of the model and the supporting experimental data were published in Sipiczki, 2018.

List of in-extenso publications describing the results of the project:

Karanyicz, E., Antunovics, Z., Kallai, Z., Sipiczki, M.: Non-introgressive genome chimerisation by malsegregation in autodiploidised allotetraploids during meiosis of *Saccharomyces kudriavzevii* x *Saccharomyces uvarum* hybrids. Applied Microbiology and Biotechnology **101**:4617-4633, 2017 doi: 10.1007/s00253-017-8274-9 IF: 3.34

Sipiczki, M.: Interspecies hybridisation and genome chimerisation in *Saccharomyces*: Combining of gene pools of species and its biotechnological perspectives. Frontiers in Microbiology **9**:3071, 2018. doi: 10.3389/fmicb.2018.03071. IF: 4.250

Sipiczki, M.: Yeast two- and three-species hybrids and high-sugar fermentation. Microbial Biotechnology **12**:1101-1108. 2019. doi: 10.1111/1751-7915.13390. IF: 5.328

Szabó, A., Antunovics, Z., Karanyicz, E., Sipiczki, M.: Diversity and postzygotic evolution of the mitochondrial genome in hybrids of *Saccharomyces* species isolated by double sterility barrier. Frontiers in Microbiology **11**:838. 2020. doi: 10.3389/fmicb.2020.00838 IF: 5.64

Sipiczki, M., Antunovics, Z., Szabo, A.: *MAT* heterozygosity and the second sterility barrier in the reproductive isolation of *Saccharomyces* species. Current Genetics **66**:957-969, 2020. doi: 10.1007/s00294-020-01080-0. IF: 3.886

Sipiczki, M.: When barcoding fails: genome chimerisation (admixing) and reticulation obscure phylogenetic and taxonomic relationships. Molecular Ecology Resources (accepted for publication) IF: 7.09