Cytological mechanism of hybridogenesis in the endangered European hybrid frog *Pelophylax grafi*

Interspecies hybrids are known in nature and in breeding. Viable hybrids of animals - unlike those of plants - are rare and most often sterile. This happens because the chromosomes from the father of one of the species and the mother of the other are too different. Mixed chromosomes can function well in cells throughout the body (somatic cell lines 2n), but not in the germline cells that form gametes (eggs and sperm 1n). Few hybrids crossed this barrier and modified reproduction, which allowed for the formation of new generations of hybrids. In other words, hybrids arise from hybrids (hybrido-genesis), and not directly from the crossing of two species. To understand the mechanism of hybridogenesis, one must study germline cells because only gamete precursors have the ability to undergo meiosis, a specific cell division that produces cells that contain only one (1n) set of chromosomes. A key stage of meiosis is the pairing of homologous chromosomes (one inherited from the father, the other from the mother), which enables the exchange of chromosome segments (recombination) and the creation of a new combination of genes. Chromosomes in hybrids are too different (homologous) and do not form the correct pairs, which leads to abnormal meiosis, and as a result, functional gametes are not formed. Hybrid reproduction is possible thanks to a very rare modification of gametogenesis, which allows to overcome these barriers. It consists of two steps: elimination of all chromosomes (genome) of one of the parental species, and then replication of the remaining chromosomes of the other species. In this way, cells recover the required number of chromosomes before meiosis, but each pair is genetically identical. Meiosis can proceed unhindered but without recombination. Genetical copies (clones) of the chromosomes of only one of the parental species are transferred to the gametes. Hybrids reproduce in the population thanks to the combination of clonal gametes of the hybrid with the gametes of the second parental species that coexist in the same population. However, when the number of individuals of one of the parental species decreases, hybrids interbreed with other hybrids, thus leading to the formation of neoform of the other species, disturbing the structure of the population and negatively affecting its genetic diversity. The edible frog *Pelophylax esculentus* is the most comprehensively studied hybridogenetic hybrid. The story began in Poland and was initiated by prof. Leszek Berger from Poznań, whose discovery attracted many researchers from different countries. All cells in this amazing frog's body have two different sets of chromosomes: one from the marsh frog (*P. ridibudnus*) and the other from the pool frog (*P. lessonae*). Our team's research has shown that genome elimination occurs in gamete precursors (gonocytes) occurring only during the early development of ovaries and testes in tadpoles (Ogielska 1994). The elimination of chromosomes takes place during interphase by forming micronuclei that bud off from the nucleus. Each micronucleus contains one eliminated chromosome (Dedukh et al. 2020), but this is an imperfect process (Chmielewska et al. 2022). Then the micronuclei are degraded by autophagy (Chmielewska et al. 2018). The water frog is not the only hybrid belonging to the genus *Pelophylax*. This taxon includes *P. hispanicus* (*P. bergeri* x *P. ridibundus*) (Uzzell and Hotz 1979) and *P. grafi* (*P. perezi* x *P. ridibundus*) (Crochet et al. 1995). The reason for attempting this particular research project is the poor degree of knowledge and complete absence of research on the mechanism of hybridogenesis in these hybrids, which opens up new research possibilities. The aim of the project is to check whether the mechanism of chromosome elimination is conservative, i.e. whether it occurs in the same way in all hybrids of European water frogs and whether, as a result of cross-breeding, it may affect the invasiveness of *P. ridibundus*. We chose *P. grafi* as the research model because we assume that gametogenesis in this hybrid is less complicated than in *P. esculentus,* in which occurs triploidy and simultaneous production of two types of gametes (containing *ridibundus* and *lessonae* chromosomes). This results in a high frequency of degenerating germline cells at every step of gonadal development. If our assumptions are confirmed, P. grafi may become a model for future research that will further explain the basis of hybridogenesis. The most important expected results are: (1) what is the composition of the germ cell genome?; (2) are micronuclei carriers of the eliminated genome in gonocytes?; (3) what type(s) of genome(s) does the hybrid pass on to the offspring?; (4) can the shortage of *P. perezi* individuals in the population lead to the generation of *P. ridibundus* offspring? We will conduct cytogenetic studies by differential staining of whole genomes in gonocytes of *P. grafi, P. ridibundus* and *P. perezi*. We will study tadpoles with known genotypes obtained through controlled crossbreeding. Then we will analyze them using cytogenetic and taxonomic techniques.