

Western Palearctic water frogs, due to their unique breeding system - hybridogenesis, constitute one of the most interesting models to study evolution and ecology of amphibians. In Central and Eastern Europe, including Poland, two water frog species: pool frog *Pelophylax lessonae* and marsh frog *P. ridibundus* and their hybrid edible frog *P. esculentus* occur. Parental species are ecologically isolated, but hybrid occur in mixed population with one of them and form different genetic systems from which the most widespread are *lessonae-esculentus* (L-E) or *ridibundus-esculentus* (R-E). The main essence of hybridogenesis is reproduction and maintenance of hybrid form by backcrosses with one of the parental species that co-occur in mixed populations. During gametogenesis, hybrids exclude one of parental genome from the germ line prior to meiosis and transmit the other one (not recombined) to the gametes. Crucial for hybrid maintenance is elimination of genome belonging to the species that is present in the mixed population (e.g. L-genome in L-E systems).

Our long-term field observations indicate that changes in the structure of water frogs mixed populations may cause the loss of stability of genetic systems. We noted that populations of *P. lessonae* are decreasing, whereas *P. ridibundus* emerges in habitats where this species has not been seen before, including habitats typical of *P. lessonae*. So far, the exact reasons of this phenomenon has been poorly studied.

The main goal of the project is to compare the species composition of L-E and R-E mixed populations at selected sites over several decades. We have comparative archival material from two regions of Poland:

1. Materials collected since the 1960 by professor Leszek Berger and his team in Wielkopolska (23 study sites).
2. Materials collected since the 1990 by our team in Lower Silesia (5 study sites).

Previously, we found the genome of the Balkan frog *P. kurtmuelleri* in Lower Silesia. *P. kurtmuelleri* probably replaces *P. ridibundus* but does not contribute hybridogenetic hybrids. Its occurrence destabilizes genetic systems which maintains due to hybridogenesis. Genetic studies on archival materials allow us to answer the question, whether *P. kurtmuelleri* was present in Poland in the past or was introduced recently, as is the case of Western Europe.

To obtain comparable data, we will use molecular methods for taxonomic identification of individuals from archival collections. For this purpose, we will use blood from dry smears, which were used to assess the ploidy of individuals in the past. The obtained results will allow us better understand the changes occurring in populations of water frogs, which form unique genetic systems. It will also enable the development of effective methods for the protection of amphibians not only at the species but also evolutionary processes levels (conservation genetics). Moreover, we will collect empirical data that may serve as the basis of new models of water frog population dynamics.