

## SUMMARY

Due to the key role of reproductive isolation in speciation, it is vital to understand the mechanisms of the emergence of barriers between diverging species and their accumulation over time. Research on species with incomplete reproductive isolation, especially those forming hybrid zones, gives valuable insights into the processes of speciation. Newts *Lissotriton vulgaris* and *L. montandoni* hybridize in nature and form hybrid zones. Historical demography, hybridization and introgression have been extensively studied in this system. This makes the species pair a good system to investigate mechanisms underlying reproductive barriers.

To extend our knowledge of reproductive isolation between these species, a genetic linkage map based on the F2 cross of *L. vulgaris vulgaris* × *L. montandoni* was constructed. This required development of suitable molecular markers. Because one of the uses of this map was localization of candidate genes, targeted resequencing was the method of choice. Molecular Inversion Probes (MIPs), used typically in biomedical and pharmaceutical researches, have been identified as a suitable approach. In the first part of thesis, the utility of MIPs in evolutionary and ecological research in non-model organisms has been evaluated using *Lissotriton* newts, which have both large and complex genome. To design the marker at least partial knowledge of the target genome is required – in this case previously identified transcript sequences were used. Genotyping the markers on genomic DNA required identification of exon boundaries, which was done using gene models of *Xenopus tropicalis*. Only 5% of the designed MIPs did not work and their specificity – the percentage of sequences that mapped to the corresponding target – reached 77% (similarly to PCR based methods on non-model organisms). Genotyping was highly reproducible (over 99%). MIPs have turned out to be suitable markers for research on non-model organisms that requires many (hundreds or thousands) samples and many (tens to thousands) short genomic regions.

The linkage map was constructed, based on two hybrid families of the F2 cross using MIPs as molecular markers. To obtain sufficient number of offspring, F2 larvae were collected in two consecutive breeding seasons. The map contains 1146 markers representing protein coding genes, located in 12 linkage groups, which most likely correspond to 12

chromosomes of *Lissotriton*. The map length of 1484 cM agrees well with the number of chiasmata observed during the meiotic division. Comparison of published maps of salamanders indicates a quick evolution of recombination rate within this group of organisms. The analysis of the loci showing departures from Mendelian segregation (segregation distortions) allowed to identify two large linkage blocks of distorted markers in two different linkage groups. The distortions occurred mostly among the offspring of one of the hybrid families during one breeding season, which suggests an environment-dependent larval mortality. Because deficit of *L. montandoni* alleles was detected in almost all distorted loci, the most likely causes may be an environment-dependent mortality of hybrids or low survival rate of larvae with *L. montandoni* alleles, based on the differences of preferred environment of both species. Both explanations indicate a possible role of the identified linkage blocks in reproductive isolation.

The last part of the thesis investigated the morphological differences between the species and hybrids, which likely contribute to reproductive isolation. The existing F1 experimental population allowed the comparison of morphological traits between hybrid males and males of parental species (including two subspecies of *L. vulgaris*: *L. v. vulgaris* and *L. v. ampelensis*) based on the developed morphological index. F1 hybrids differ significantly from the other groups and their phenotype is intermediate between the parental species. The spots on the dorsal side, an important trait in identification of *Lissotriton* species, have been measured quantitatively for the first time. The morphological index used on two transects through the hybrid zone indicated the hybrids of *L. v. ampelensis* × *L. montandoni* are indistinguishable from *L. v. ampelensis*, which may affect the strength and direction of sexual selection among hybrids and parent species as the sexual attractiveness of hybrid males may be similar to *L. v. ampelensis* conspecific. Further research should include the location of loci underlying variation in morphological traits (by QTL analysis) on the genetic linkage map and comparing them to the heterogeneous gene flow, as traits linked with genomic regions of decreased/increased gene flow may play important role in partial reproductive isolation between species, especially by influencing sexual selection.

