

MASTER BIOINFORMATIQUE

Master Internship offer M2

Internship supervisors and host laboratory

Supervisors: Carina Farah Mugal (CR CNRS, carina.mugal@univ-lyon1.fr), Anamaria Necsulea (CR CNRS, anamaria.necsulea@univ-lyon1.fr)

Host laboratory: Laboratoire de Biométrie et Biologie Evolutive (LBBE), 43 boulevard du 11 novembre 1918, Villeurbanne <https://lbbe.univ-lyon1.fr/>

Research project title

Investigation of the role of splicing variants in the context of speciation

Project description

Speciation is a central evolutionary process that can be described as the formation of reproductively isolated species, where the build-up of reproductive barriers can originate from different routes (Figure 1). Reproductive isolation can be initiated by divergent selection on ecological traits or mating preferences, or originate from incompatibilities between two or more loci that interact with each other, for example during the formation of the zygote or also in the genomic background of the hybrid (Coyne & Orr 2004; Presgraves 2010). In order to establish the route of reproductive isolation and improve our understanding of speciation, one of the major objectives in speciation research has therefore been directed towards pinpointing the genetic loci that encode the reproductive barriers. Two classical candidates that have received much attention are evolutionary changes in protein-coding sequences as well as changes in their expression levels. The role of splicing variation remains, on the other hand, largely unexplored.

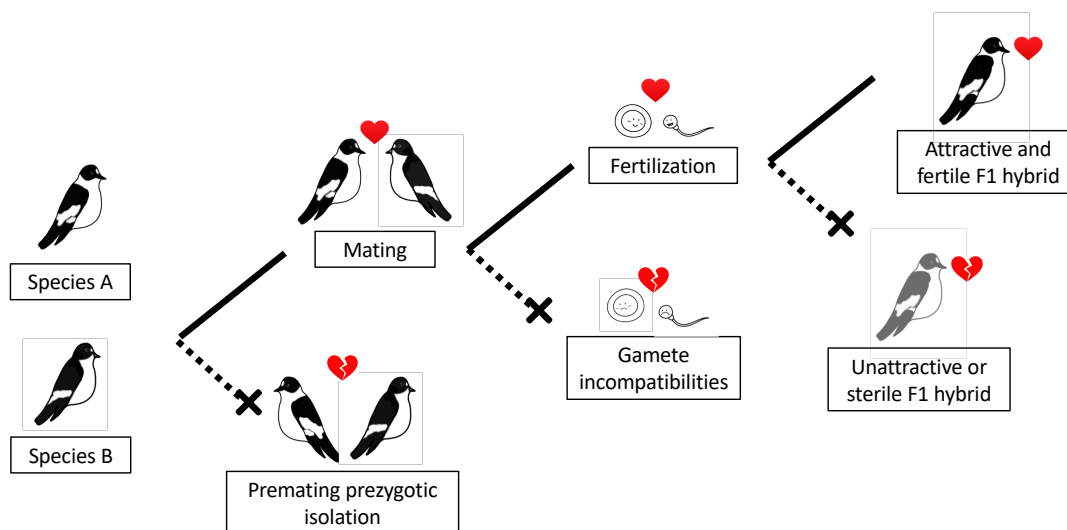


Figure 1: Schematic illustration of different stages of reproductive isolation along the reproductive cycle, i) premating prezygotic isolation, which e.g. can result from geographic isolation or assortative mating, ii) postmating prezygotic isolation, such as gamete incompatibilities, and iii) reduced hybrid fitness due to either extrinsic environmental factors or intrinsic genomic hybrid incompatibilities.

In eukaryotic species, splicing is a fundamental process through which introns are excised out from precursor messenger RNA and exons are spliced together to generate mature RNA molecules. Splicing makes genes modular allowing for several splice variants (or alternative splicing) of the same gene, which can be observed at many levels: within a single cell, among different cell types or tissues within an organism, among individuals of the same species or among different species. These splice variants are increasingly acknowledged as potentially playing an important role in phenotypic evolution and adaptation (Verta & Jacobs, 2022). In addition, incompatible splice variants among diverging species could represent a molecular target for reproductive isolation and speciation.

The project will focus on the characterization of splice variants in two closely related *Ficedula* flycatchers, which are well-known species in the field of speciation research. Collared flycatchers (*Ficedula albicollis*) and pied flycatchers (*Ficedula hypoleuca*) diverged approximately 1 MYA but co-occur in natural hybrid zones such as the Swedish island of Öland (Saetre et al. 1999; Qvarnström et al. 2010). Their F1 hybrids suffer from low to totally compromised fertility (Alatalo et al. 1990; Ålund et al. 2013), low attractivity (Alatalo et al. 1982; Svedin et al. 2008), and abnormal metabolic rate (McFarlane et al. 2016). Differential expression analysis of protein-coding genes provides candidate genes that could explain the observed abnormal metabolic rates in hybrid individuals, but fails to explain their compromised fertility (Mugal et al. 2020). Here, the availability of PacBio long-read isoform sequencing (IsoSeq) data across multiple tissues, both sexes and from both species provides an excellent opportunity to complement earlier findings by the study of splice variants across tissues, sexes and species and investigate their role in reproductive isolation and speciation.

Specific tasks

- Characterization of splice variants based on PacBio long-read isoform sequencing data
- Quantification and differential splice variant analysis among tissues, sexes and species
- Identification and functional annotation of candidates of splice variants between collared flycatchers and pied flycatchers that could contribute to reproductive isolation

As part of the project work, the student will develop skills in handling high-throughput sequencing data and statistical data analysis with R. Specifically, the student will get familiar with computational methods for the analysis of long-read transcriptome data (e.g. minimap2, TAMA, StringTie2) as well as popular tools for differential splice variant analyses (e.g. KissSplice, DEXSeq and LeafCutter), and will gain a sound understanding of genome annotation and the analysis of expression patterns, which are skills central to functional genomics, comparative genomics and speciation genomics.

Expected profile and skills of the candidate

The candidate should have a strong interest in evolutionary biology; should be willing to acquire an extensive training in bioinformatics and statistical data analysis; should have good communication skills in English and enjoy collaborative work.

This work will be conducted in the context of an international long-term collaboration on *Ficedula* flycatchers between David Wheatcroft (Stockholm University, Sweden), Anna Qvarnström (Uppsala University, Sweden), Anamaria Necsulea (University of Lyon 1, France) and Carina Mugal (University of Lyon 1, France).

Relevant publications from the team

- Bénitère F, Necsulea A, and Duret L. 2023. Random genetic drift sets an upper limit on mRNA splicing accuracy in metazoans. *bioRxiv*. doi: 10.1101/2022.12.09.519597
- Mugal CF, Wang M, Backström N, Wheatcroft D, Ålund M, Sémon M, McFarlane SE, Dutoit L, Qvarnström A, and Ellegren H. 2020. Tissue-specific patterns of regulatory changes underlying gene expression differences among *Ficedula* flycatchers and their naturally occurring F1 hybrids. *Genome Res* 30:1727-1739. doi: 10.1101/gr.254508.119.
- Qvarnström A, Rice AM, and Ellegren H. 2010. Speciation in *Ficedula* flycatchers. *Philos Trans R Soc Lond B Biol Sci* 365:1841-1852. doi: 10.1098/rstb.2009.0306.

References

- Alatalo RV, Eriksson D, Gustafsson L, and Lundberg A. 1990. Hybridization between pied and collared flycatchers - sexual selection and speciation theory. *J Evolution Biol* 3:375-389. doi: 10.1046/j.1420-9101.1990.3050375.x
- Alatalo RV, Gustafsson L, and Lundberg A. 1982. Hybridization and breeding success of collared and pied flycatchers on the island of Gotland. *Auk* 99:285-291. doi: 10.1093/auk/99.2.285
- Ålund M, Immler S, Rice AM, and Qvarnström A. 2013. Low fertility of wild hybrid male flycatchers despite recent divergence. *Biol Letters* 9:20130169. doi: 10.1098/rsbl.2013.0169
- Coyne JA, and Orr HA. 2004. Speciation. *Sinauer Associates*, Sunderland, MA.
- McFarlane SE, Sirkiä PM, Ålund M, and Qvarnström A. 2016. Hybrid dysfunction expressed as elevated metabolic rate in male *Ficedula* flycatchers. *PLoS One* 11:e0161547. doi: 10.1371/journal.pone.0161547
- Mugal CF, Wang M, Backström N, Wheatcroft D, Ålund M, Sémon M, McFarlane SE, Dutoit L, Qvarnström A, and Ellegren H. 2020. Tissue-specific patterns of regulatory changes underlying gene expression differences among *Ficedula* flycatchers and their naturally occurring F1 hybrids. *Genome Res* 30:1727-1739. doi: 10.1101/gr.254508.119.
- Presgraves DC. 2010. The molecular evolutionary basis of species formation. *Nat Rev Genet* 11:175-180. doi: 10.1038/nrg2718
- Qvarnström A, Rice AM, and Ellegren H. 2010. Speciation in *Ficedula* flycatchers. *Philos Trans R Soc Lond B Biol Sci* 365:1841-1852. doi: 10.1098/rstb.2009.0306.
- Saetre GP, Kral M, Bures S, and Ims RA. 1999. Dynamics of a clinal hybrid zone and a comparison with island hybrid zones of flycatchers (*Ficedula hypoleuca* and *F. albicollis*). *J Zool* 247:53-64. doi: 10.1111/j.1469-7998.1999.tb00192.x
- Svedin N, Wiley C, Veen T, Gustafsson L, and Qvarnstrom A. 2008. Natural and sexual selection against hybrid flycatchers. *Proc Biol Sci* 275:735-744. doi: 10.1098/rspb.2007.0967
- Verta JP, and Jacobs A. 2022. The role of alternative splicing in adaptation and evolution. *Trends Ecol Evol* 37:299-308. doi: 10.1016/j.tree.2021.11.010