Summary

Context

Since Darwins' "The Origin of Species" there has been a continued interest in the speciation process. Adaptive radiations, such as those found in cichlid fish, have for decades proven to be ideal model systems to study this. Indeed, the study of cichlids provided valuable insight in the geographical, ecological and behavioural mechanisms that drive speciation.

The development of novel sequencing technologies has given a new impetus to this field, especially since annotated cichlid genomes became available. To date, recently developed techniques that allow exploring genomic evolutionary issues in cichlids have for the most part been used to link genes with the observed phenotypic changes that are so characteristic in the African cichlid species flocks. However, much fewer studies have tried to apply the genomic toolbox to the study the genetic basis of cichlid behaviour. As several of the cichlid species in the East African Great lakes co-exist and can easily hybridise, behavioural mechanism are thought to be among the main drivers to the speciation process.

Objectives

We have studied species boundaries using a small genus of Lake Tanganyika cichlids, *Ophthalmotilapia*, as a model. For this genus, the different species: *O. nasuta, O. boops, O. ventralis* and *O. heterodonta*, are known to co-exist and hybridise in the wild. *Ophthalmotilapia* species all have pronounced sexual dimorphism. They are maternal mouthbrooders and display a complex and elaborate mating ritual. For these three reasons, we expected that female mate choice is responsible for maintaining species boundaries. Interestingly, hybridisation among *Ophthalmotilapia* species was shown to be asymmetrical. This implies that females of one species were less inclined to accept heterospecific mates than females of the other species. This allows us to not only investigate species boundaries, but also to compare species boundaries that differ in strength. We have combined a genomic, transcriptomic and behavioural toolbox to investigate how these species boundaries are maintained in this genus. Additionally, we have attempted to identify so called 'speciation genes', genes that are responsible for driving the speciation process. By screening the genomes of species in the genus across its entire distribution range, we have explored whether these potential speciation genes also contributed to the diversification of the genus, and to the maintenance of species boundaries in nature.

Methodology

We have combined a 'classical' behavioural study with a comparison of the transcriptome of the cichlid brain during and prior to mating. For this, we conducted two sets of behavioural experiments that describe the initial and the final phases of the mating process: a brief first encounter with a potential mate, and an entire mating event that ended with a successful reproduction. The experiments were performed using wild-caught *O. nasuta* and *O. ventralis* specimens, originating from a locality in Lake Tanganyika were they occur in sympatry. Both experiments were performed in a con- and a heterospecific setting, i.e. female specimens of *Ophthalmotilapia* were presented with con- or a heterospecific male. The behaviour of females was compared in an unbiased way. After the experiments, the differential gene expression from different brain parts were used for RNA sequencing. Transcriptomes of the different brain parts were compared for specimens subjected to a different treatment and up- and downregulated genes were identified, using the annotated genome of the Nile tilapia. We also adopted a genotyping by sequencing approach was followed to obtain SNP

data for 500 samples of *Ophthalmotilapia* and related species, which allowed us to investigate patterns of hybridisation.

Results

Given the patterns of asymmetric hybridisation observed in nature, we predicted that females of *O. ventralis* would be less discriminatory towards heterospecific males than females of *O. nasuta*. This was confirmed in the presentation experiments were we observed species recognition for females of *O. nastra* but not for those of *O. ventralis*. The formed behaved differently when observing a con- or a heterospecific male, whereas for the latter, no significant difference in behaviour was observed. When looking at neural gene expression, fourteen genes were differentially expressed in at least one of the brain parts of *O. nasuta* females after seeing an *O. nasuta* or an *O. ventralis* male. The lack of discriminatory behaviour in female *O. ventralis* was also observed in the mating experiments. Here, three successful mating events were observed between an *O. ventralis* female and an *O. nasuta* male. The comparison of the transcriptomes of *O. ventralis* females after con- and heterospecific mating, revealed that 106 genes were differentially expressed. Phylogenetic and phylogenomic analyses revealed similar geographic structure in *Opthalmotilapia*. Genetic structuring analyses revealed that currently valid species were supported. However, instances of hybridisation, previously only found with mitochondrial markers were also retrieved using a GBS approach.

Conclusions

The earlier reported hybridisation among *Opthalmotilapia* species - based on a few genetic markers - could be confirmed with a genomic approach. Mating experiments revealed that the patterns of asymmetric hybridisation seen in nature are due to differences in species recognition and mate choice in the females and not because of differences in mating preferences of the males. Differential gene expression in different brain parts also indicate that only females are able to differentiate between con- and heterospecific males. Additionally, differential gene expression occurred in the same brain parts and as similar biological processes were involved in species recognition in both experiments. We predict that these genes, which can be considered candidate speciation genes, modulate processes that might inhibit hybridisation under natural conditions.

Key words: Speciation, Behaviour, Mate choice, Transcriptome, Brain