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Characterizing the dynamics of stationary vegetation boundaries

Csaba Tölgyesi

Department of Ecology, University of Szeged, Szeged, Hungary

The vegetation of landscapes consists of patches and intervening boundaries. Boundaries are often considered as the hotspots of vegetation changes, since several plant species reach the limit of their local distribution at the boundaries. Therefore, the dynamics of boundaries can be a sensitive indicator of environmental changes and can help to understand the mechanism of landscape scale pattern changes.

The dynamics of boundaries is usually described by their positional movements. Positional boundary dynamics has three main types: stationary, directional and shifting. A boundary is stationary, i.e. stable in position, if strong and stable abiotic parameters maintain them over long periods of time. Directional dynamics means the unidirectional translocation of boundaries due to abiotic or biotic drivers, while shifting dynamics takes place if these factors fluctuate in time.

In my study, I aimed to refine this scheme by analysing the dynamics of two vegetation complexes. The first one was located in Bugacpuszta, a landscape dominated by sand dune ranges with xeric plant communities on dunes and mesic ones in dune slacks. The other location was the Turjánvidék, which is a mosaic of wet meadows and steppe fragments. In both cases, the emergence of boundaries is dependent on topography-driven differences in water supply; therefore stationary boundary dynamics is expected to prevail. However, in the first case the mesic patches gradually lost the majority of their water supply in the last few decades, meaning that a key environmental factor had a directional trend. In the Turjánvidék, the average water supply is still satisfactory, but its inter-annual variations have become much larger, which can be considered as a shifting effect.

In Bugac, the vegetation of a 55-m and a 30-m transect were monitored between 1999 and 2013 using contiguous grids of quadrats. Presence/absence data of all plant species was recorded and the data were analysed with the split moving window technique. The method identifies the position of boundaries and the contrast the boundaries bridge between the patches. Five significant boundaries could be detected along the transects over the years. I tested the dynamics of their position and contrast using linear regressions. According to the results, positions did not follow the retraction of the groundwater, but the contrast between the adjacent patches showed significant trends: two boundaries disappeared and one new one appeared, which could be explained by the increasing proportion of xeric species and by the opening up of the vegetation.

In the Turjánvidék, I surveyed the vegetation along thirteen permanent, 40 m long transects. The surveys were performed in a wet year (in 2013) and in a dry year (in 2014). After performing the split moving window analysis, the boundary parameters were compared between the two years using t-tests. The results indicated that the position of the boundaries did not react to the differences of water availability, but the wet and dry patches became more similar to each other in the dry year, which could be traced back to the higher proportion of xeric species in the wet patches in 2014.

Thus, it can be concluded that even strong directional or shifting environmental processes cannot elicit positional changes in stationary boundaries; therefore positions are not recommended for bioindication in these cases. On the other hand, contrast changes sensitively indicated the reactions of the vegetation. This also means that landscapes with stationary boundaries can also be dynamic, but the typical pattern changes are not patch expansion vs. shrinking but patch fusion and division, which should be taken into consideration in a variety of ecological and nature conservation applications.

Supervisor: László Körmöczi
E-mail: festuca7@yahoo.com

Genetic investigations in neurodegenerative disorders

Nóra Török

Department of Neurology, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary

Genetic investigations are useful tools to identify genes, which directly or indirectly play a role in the pathomechanisms of diseases and thereby indicating useful therapeutic interventions. The aim of our research group was to investigate different single nucleotide polymorphisms (SNPs) in multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). As a first step a biobank in the Department of Neurology, Szeged was established. Necessary ethical and biobank licenses were obtained and sample collections were started. Parallel with the collection of blood samples, databases belonging to the samples (age, sex, disease onset, etc.) were created. DNA purification from whole blood was performed using a simple salting out technique developed by Miller. To distinguish the alleles of the examined genes fluorescent labeled Taqman probes or restriction fragment length polymorphism techniques were applied. For statistical evaluation of the data SPSS software version 20 was utilized.

In case of MS chemokine receptor V polymorphism rs333 was studied, since earlier results about its involvement in the pathomechanism of MS were controversial. We investigated the receptor and its deletion in a large MS (428) and control (831) population from Hungary and Serbia. There was no significant difference in genotype (OR=1.092, 95% CI=0.807-1.478, p=0.568 for wt/wt (wt=wild type allele) vs wt/Δ32, Δ32/Δ32 (Δ32=Δ32 base pair deletion allele)) or allele frequency (OR=0.914, 95% CI=0.692-1.207, p=0.525). Neither the deletion

nor the wt allele affected the Expanded Disability Status Scale score or the age at onset. Our results indicate no association between the chemokine receptor V Δ32 allele and MS.

For the investigation of pathomechanism of PD two different genes were analysed, vitamin D receptor which encodes a transcription factor that influences calcium homeostasis and immunoregulation, and the kynurenine-3-monooxigenase, which is the key enzyme of the kynurenine pathway.

In the vitamin D receptor study 100 PD patients and 109 healthy controls from the Hungarian population were genotyped for four polymorphic sites (BsmI, ApaI, FokI and TaqI). Our results demonstrate an association between the FokI C allele and PD, since the frequency of the C allele was significantly higher in PD patients than in controls, suggesting that this polymorphism may have a role in the development of PD in Hungarian patients.

There is substantial evidence that the kynurenine pathway plays a role in the normal physiology of the brain and it is involved in the pathology of neurodegenerative disorders. 105 unrelated, clinically definitive PD patients and 131 healthy controls were enrolled to investigate the possible effects of the different alleles of kynurenine-3-monooxigenase. None of the four investigated SNPs proved to be associated with the disease or with the age at onset. The investigated SNPs presumably do not appear to influence the gene function and probably do not contain binding sites for regulatory proteins. This was the first study to assess the genetic background behind the biochemical alterations of the kynurenine pathway in PD, directing the attention to this previously unexamined field.

Evidences indicate that there are aberrations in vitamin D endocrine system in ALS too. Our aim was to investigate SNPs from vitamin D receptor gene in 75 sporadic ALS patients and in 97 healthy controls. One of the four investigated SNPs was associated with the disease, but none of the alleles of the four examined SNPs influenced the age at disease onset. The ApaI A allele was more frequent in the ALS group compared to the control group, so it may be a risk factor for getting the disease.

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Supervisor: László Vécsei
Email: vecsei.laszlo@med.u-szeged.hu

Identification of novel genes involved in the virulence of *Candida parapsilosis* during the generation of deletion library

Renáta Tóth

Candida Research Group, Department of Microbiology, University of Szeged, Szeged, Hungary

Recently, the increase in the prevalence of fungal diseases has focused attention on understanding the interactions between the pathogens and the host. Despite the growth of sequence information, a large number of fungal genes are uncharacterized and the function of genes are based solely on sequence homology. To characterize gene function in fungi such as the opportunistic pathogen *Candida parapsilosis*, gene knockout methods can be applied. In our previous work we have identified several fungal genes using RNA-Seq data that were overexpressed during host-pathogen interactions. To investigate their functions the creation of a knock out library was prepared. We have adapted a gene knock out strategy from the work of Noble and Johnson (2005). Fusion PCR method was applied to generate gene specific deletion constructions in order to disrupt genes from the genome of *C. parapsilosis* CLIB leu-/his- auxotrophic strain. Primarily we generated the flanking PCR products for the upstream and downstream regions for each of the genes, and the HIS1 and LEU2 marker PCR products. We used HIS1 marker from plasmid vector pSN52, and LEU2 from plasmid vector pSN40. Transformation of *C. parapsilosis* cells was performed chemically, using polyethylene glycol. For each of the identifications we used colony PCR to confirm the total deletion of the genes. All of the mutants were barcoded using a 20 bp tag in order to be able to identify them during later *in vivo* infections. All of the null mutant strains were tested under different conditions such as growth abilities on certain temperatures and medias, survival in the presence of cell wall, osmotic and oxidative stressors, and also pseudohyphae formation. Resistance to antifungal drugs such as fluconazole and caspofungin was also examined. We found null mutants that showed differences in appearance such as increased pseudohyphae formation and resistance to cell wall stressors (CPAR2 200390), regressed growth on different temperatures (CPAR2 303700) and alkali-phobic phenotype (CPAR2 100540). Difference in the virulence of these null mutant strains was also found when using infection models. CPAR2 200390 null mutants were found to be killed less efficiently by murine macrophages *in vitro*. In contrast, null mutants of CPAR2 303700 were killed similarly, however phagocytosed less than wild type cells by macrophages. Furthermore, more murine macrophages were found to phagocytose CPAR2 100540 deletion mutants, however the killing efficiency was lower comparing to the reference strain. In the future using this method we will be able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.

References

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