Expert meeting: Hannes Svardal

Location: RBINS (Brussels)

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14/12/2017

Present: Erik Verheyen, Sofie Derycke, Loic Kéver, Koen Herten, Jos Snoeks, Maarten Van Steenberge, Erik Parmentier, Gregory Maes, Alvaro Cortes Calabuig, Koen Van den Berge, Jonas Lescroart

Excused: /

Acronyms: Royal Belgian Institute for Natural Sciences (RBINS); Royal Museum for Central Africa (RMCA); University of Liège (ULg); University of Leuven (KUL); Belgian Science Policy (BELSPO); *Ophthalmotilapia ventralis* (OV); *Ophthalmotilapia boops* (OB); *Ophthalmotilapia nasuta* (ON)

10.00h – 11.15h: Seminar by Hannes Svardal: *Ancestral hybridisation facilitated species diversification in the Lake Malawi cichlid fish adaptive radiation* (open to all interested scientists). In this talk, Hannes presented his work at the Sanger institute and at the University of Cambridge where they investigate the genomic basis of the rapid speciation in Lake Malawi cichlids (Malawi cichlid genome project). Given the importance of hybridisation in the early stages of the radiation in Lake Malawi, this work has a strong link with the research questions of the GENBAS project.

11.30h-12.00h: Presentation GENBAS project (GENBAS partners only); overview of the aims of the project

12.00h-15.15h: Presentations on progress of each work package and planning of future actions

1/ New composition of the GENBAS team

- Sofie Derycke has taken up a position at ILVO since 6-2017 and Maarten Van Steenberge has taken over her position at the RBINS from 6-2017 until 3-2018 (part time) and from 4-2018 until 3-2019 (full time).
- At the University of Liege, an MSc will be hired for a period of 6 months. This person will have a background in ethology and will conduct additional experiments for the GENBAS project.
- At the RBINS, a post-doc will be hired for a periods of 1 year. This person will, preferably, have a background in bioinformatics and NGS.
    - 50 applications were received. A preliminary shortlist of the top 5 candidates has been compiled (Erik and Maarten). CV's are available in a shared dropbox folder, and suggestions to this shortlist are welcome. At the 2nd of January, five applicants will be informed that they will be invited for an interview on the 5th of January. The contract should start in February.

2/ Timeline of the GENBAS project

- A quick overview of the different work packages (WP) as laid forward in the project proposal is presented.
- WP1: paring experiments
  - All tasks related to this WP are done:
    - ⇨ Behavioural experiments have been performed at ULg
    - ⇨ As acoustic communication did not prove to be important in *Ophthalmotilapia*, experiments did not focus on acoustics solely.
    - ⇨ Ethograms were made for OB, ON and OV.
    - ⇨ Manuscript has been accepted in *Zoology*
    - ⇨ Additional experiments (post- and pre-mating) performed at ULg.
      - data analysed, might need additional replicates (see below)
- WP2: Sample preparation for GBS and RNA Seq
  - All tasks related to this WP are done
    - ⇨ Samples for RNA seq from three experiments were prepared
    - ⇨ DNA was extracted from over 400 samples, DLoops were sequenced and included in a dataset that contains 515 sequences.
    - ⇨ 404 samples were prepared for GBS, including all representatives of the tribe Ectodini
- WP3: Next generation Sequencing:
  - Task related to this WP are ongoing
    - ⇨ RNA was sequenced from data from three different experiments
      - The analysis of the data revealed run effects (see below)
    - ⇨ GBS has been performed on 404 samples in three different pools
      - Here too, a run effect was present in the data (see below)
- WP4: Data analysis genomics
  - Task related to this WP are ongoing
    - ⇨ Data analysis RNA seq
      - Manuscript on RNA seq (pilot study) will be resubmitted after addressing the reviewer comments (*Frontiers in Neuroscience*)
      - Run effects were found that hampered the integration of the different datasets
    - ⇨ Data analysis GBS
      - Preliminary analyses were performed on pool1
      - The analyses of the samples on all three pools was hampered by what seemed to be a run effect.
  - WP5: Data Integration behaviour and genomics
    - ⇨ Task related to this WP are ongoing
      - behavioural and RNA seq data collected during the PrM and PoM experiments are being analysed
      - integration of RNA seq and GBS remains to be done.
  - WP6: Network coordination and dissemination
    - ⇨ Task related to this WP are ongoing

Discussion of the main experiments and analyses

3/ Pilot study

- RNA sequencing was performed on six brain parts obtained from ON and OV females that were kept in a control setting (one species, only females).
- Manuscript on will be resubmitted after addressing the reviewer comments (*Frontiers in Neuroscience*)

4/ Pre-mating experiment

- **Aim:** investigate the behaviour and the neural gene expression of a focal female of ON after being presented to either a male of ON (N=4), of OB (N=2) or of OV (N=3), to a female of ON (N=3) (all non-focal individuals) or to nothing (N=3).
- **Set-up:** Specimens were put in experimental tanks one night before the onset of the experiment. Tanks consisted of two halves that were separated by a transparent wall and a visual separation. Behaviour of the focal and the non-focal individual was recorded 15 before and 45 minutes after the visual barrier was removed. Behavioural data consisted of tracking data (coordinates) and a qualitative recording of behaviour. After 45 minutes, focal individuals were euthanized, the six brain parts were dissected and RNA was sequenced from all of them.
- **Behavioural data:**
  - both the tracking data and the qualitative data revealed interesting patterns. However, due to the limited sample size, only one behaviour revealed to be significant: when a fish (male or female) was presented to the focal individual, the focal individual approached the separation wall more than when no other fish was presented.
  - An exploratory analysis of the data revealed that focal females responded differently to the males of different species and suggested some mirroring behaviour.
  - One of the OV males performed courtship behaviour. This complicated the interpretation of the behavioural data.
- **RNA seq:**
  - The exploratory analysis revealed a clear division between brain parts, but not a strong division between treatments, except in the Diencephalon.
  - Three different contrasts were tested (GLM):
    - ⇨ Difference between control and other treatments (fish vs. no fish)
    - ⇨ Difference between seeing a con- or hetero-specific male
    - ⇨ Difference between seeing a conspecific female or male
  - It was suggested to base to let the choice of contrasts to test depend on the exploratory analyses; where behaviour of focal females presented to OV differed from that of all other focal females

    - As one of the OV males performed courtship behaviour, expression levels in the brain of the female that was presented to him (**ON38**) should be examined separately.
- **Additional experiments:** The possibility to perform additional experiments was discussed. These can be prepared by the new staff member from the ULg and performed together with the two additional staff members from the RBINS. Here, we will increase the number of replicates to five for all treatments, and also include treatments that include focal female OV instead of only focal female ON.
    - For these experiments, we decided that no extra RNA sequencing needs to be done.
    - Life fish are still available at the RBINS although additional life fishes might still need to be ordered.
- **Expected output:** Although the results of this study should be combined with those of the post-mating experiment, it should also be published independently first

5/ Post-mating experiment

- **Aim:** investigate the behaviour and the neural gene expression of a female after mating with a con- or heterospecific male.
- **Set-up:** females and males were kept in tanks until a mating event occurred. When this happened, the video of the mating was analysed in a standardised way. After mating was completed, the female was euthanized, the six different brain parts were dissected out and processed for RNA sequencing.
- **Combinations:** ON-ON (N=4); OV-OV (N=3); OV-ON (N=3),
- **RNA seq:**
    - The control samples were run on a first batch whereas the different treatments were run on batches two and three.
    - A strong batch effect was retrieved when analysing the samples.
        - ⇨ The strongest effect was seen between the control samples (pilot study, batch one) and the post-mating samples (two and three), this was evident in the difference in read mapping. Hence, the controls cannot be compared with the samples of the experiments.
        - ⇨ Also in the post-mating samples, a batch effect remained present, but this was smaller.
    - How can these effect be removed? Should some samples be rerun?
        - ⇨ Check at KUL whether this was caused by a change in protocol
            - Was there a change in the kits used?
            - Was there a change in the pipeline?
        - ⇨ Additional funds are still available at KUL for resequencing
            - This could be done at a price of €1,500 per plate (if barcodes can be combined)
            - €10,000 is still available at KUL

        - Would there be a possibility of combining the pre-and post-mating data for RNA seq?
        - For 80% of the samples, enough extract is still available
    - **Preliminary results:** DE genes were found between con- and heterospecific matings in OV (mostly in diencephalon) and between conspecific matings in OV and ON.
    - **Expected output:** The results will be combined in one paper together with the conclusions of the pre-mating experiment.

5/ Genomic data analysis

- **GBS dataset:**
    - The GBS was performed for 420 samples included in 3 runs.
    - A strong run effect was retrieved and the data on the last run could not be analysed together with that on the first and the second run.
    - It will be checked -> KUL; whether this was due to the analysis or due to a difference in sample preparation.
- **Phylogeography of *Ophthalmotialpia* and *Cyathopharynx***
    - **Aim:** delineate the lineages within these two genera using both a mitochondrial and a GBS approach.
    - **Intermediary results:** A mitochondrial haplotype network was constructed and phylogeographic analysis, based on MtDNA, were performed (eg. AIS, SAMOVA, Baysian Skyline plots…)
    - Once the GBS dataset is completed, this study will be completed with a phylogeny of the two genera.
- **Pylogeney of the Ectodini**
    - The complete GBS dataset contains representatives of all members of the tribe Ectodini.
    - KUL -> Outgroups would need to be added *in silico*: eg. *M. zebra, N. brichardi*
- **Genomic differentation**
    - When the complete dataset is available, differentiated regions in the genome will be identified between sympatric hetero-specific and allopatric conspecific populations of *Ophthalmotilapia*.
    - A subset of this dataset will be analysed by Jonas Lescroart (MSc thesis)

Meeting ends at 15.15h.