GENBAS Follow up meeting 6

Locations: Genomics Core (Leuven), Aquarium-Museum (Liège)

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11/09/2018 – 19/09/2019

Two separate GENBAS meetings were held in September 2018: one focussing on the genomic analyses (Leuven, 11-09-2018), and one on the behavioural experiments. (Liege, 19-09-2019)

Meeting Leuven

Present: Erik Verheyen, Sophie Gresham, Koen Herten, Gregory Maes, Maarten Van Steenberge

GBS dataset

When using the methodology and the pipelines that sucessfully worked on the data from the first and the second pools, problems were encountered when analysing the data from all three pools. Here, the dataset contained a lot of missing data, regardless of the filtering and pruning parameters used. The hypothesis on why the combined dataset was difficult to analyze was that pool 3 contained less samples (121) than pool 1 and pool 2 (192). Hence, pool 3 contained 30% more data for each sample. By not taking this into account in the genotyping, too many sites were lost after filtering. This also explains why there was less missing data in the 3rd plate after filtering and/or pruning.

Koen will re-run freebayes on the dataset, this was expected to be finished within two weeks. After this, Sophie will go to the core to look at the dataset and to start the analysis together with Koen. The time needed for regenotyping, was, however, somewhat underestimated and at the time of writing (9-10-2018), this analysis was still running on the cluster at the genomics core. To speed up the genotyping, it was decided to redefine regions of interest.

After basecalling, we expect anther two weeks for filtering and two weeks for analysing the data. Hence, it was expected to have a full picture of the dataset by mid-November. Yet, it seems that more time was needed for genotyping than the originally foreseen two weeks.

Some remarks were made regarding the ordering of the filtering steps. The following order was proposed: biallelic SNPs, indels, non-reference alleles, max-missing genotypes 50%, depth 5, max missing again (50-90). In the final filtering step, the setting –minGQ = 30 was lost likely too high, this should better be set to 20, or omitted completely

Especially for the entire dataset, some of the steps in the filtering pipeline should be omitted. Eg. indels might be relevant when studying the above species level.

An additional analysis was also already started in which a pooled dataset filtering was performed. Here, only variants that were present in each species were kept. Hopefully this will yield even more common fragments to do phylo on.

For phylogeny reconstruction, it was also suggested to use a Present/Absent approach (similar to AFLP) of SNP's. This approach is also followed by Frederico and Hendrik in Leuven, and we can ask them for advice when we following this approach.

RNA study

RNA sequencing has been performed on the brain parts collected from the first round of pre-mating (contact) experiments. Yet additional experiments will be performed:

a) the additional experiments focusing on ON females (N= 8) (performed at 28-30/8/2018)

b) the mirror experiments focusing on OV females (N= 20) (planned 10 -> 19 10/2018)

If we would like to sequence transcriptomes for all 6 brainparts for all of these experiments, we would need to redo. Hence, this would mean sequencing: a) 8*6= 48 samples and b) 20*6=120 samples. These could be put on a) ½ lane and b) 1.5 lanes and would cost a) €6,000 and b) €18,000. For this, we will check how much money is still available for sequencing projects.

Meeting Liège

Present: Erik Verheyen, Sophie Gresham, Noémie Jublier, Pascal Poncin, Eric Parmentier, Maarten Van Steenberge

Excused: Jos Snoeks

Changes in staff composition

Noémie started mid-May 2018 and will have a contract until mid-January 2019.

Sophie started May 2018 and will have a contract until the end of March 2019.

Work packages and experiments

A brief summary on the progress of the different work packages was presented. For this, we divided the project into three parts:

A) The genomics and genetics work.

The conclusions of the meeting at the genomics core in Leuven were summarised.

B) The post-mating experiments.

These experiments are finished and the results were briefly summarised.

C) The pre-mating experiments.

In these experiments, we will investigate the behaviour and the gene expression of a female *Ophthalmotilapia* when presented with a con- or heterospecific male, with a female or with nothing (control). Females (ON/OV) were be presented to: a) Nothing (Controle); b) a female OV; c) a male OV and d) a male OV. Specimens will be placed in the experimental tanks overnight. Here, they are separated by an opaque and transparent wall. The next day, the recordings will start. After 15mins, the opaque wall will be removed. 45 mins later, the focal female will be caught and euthanized. For this specimen, the separate brain parts will be stored in RNAlater, as well will the gonads and the gills. Length and weight of the focal and the presented individual will be taken.

Experiments have already been done, using ON as the focal species, and with 3 to 4 replicates per setting. Three types of data were collected:

- RNA sequence data for each of the six individual brain parts separately
- qualitative behavioural data
- quantitative behavioural data (tracking data)

The RNA sequencing revealed differences in gene expression between females that were presented with con- or hetero-specific males. Yet, due to the limited number of replicates, almost none of the differences in behaviour proved to be significant. Hence additional experiments were performed (28,29,30.8.2018) to bring the number of replicates per setting to 5. (ON experiments)

Additionally, a complete mirror experiment will be performed using OV as the focal species, and performing 5 replicates for each experiment. (OV experiments)

ON experiments

These were performed in Liege at 28,29 and 30-8-2018.

The tanks used in this round of experiments had slightly different dimensions than the tanks used in the previous round of experiments. This will need to be taken into account.

2 ON female specimens are left, these will be used in a following round of experiments. To be decided when we will have analysed the data.

Sophie and Maarten are analysing the video's using the tracking software; Noémie is analysing the videos using the same protocol as Loïc did. For comparison, she has also re-analysed the videos of the previous round of experiments in order to check whether she used the same criteria for scoring behaviour. Raw data should be collected by mid October.

OV experiments

New specimens arrived: these are 27 females of OV from Kala and 5 OV males from Kala. The ON males that are needed for the experiment will be the one used in the ON experiment.

The Dloop sequence of all of the new specimens will all be sequenced to verify their origin.

The experiments will be performed in the latter half of October (10-30 October)

In order to measure to which degree potential clues might be transported in the water overnight, we suggest performing a test in which a dye is put in one side of the tank, and its concentration is measured at the other side at regular intervals of time, during 12h.

The colour of the opaque wall (bright white) hinders the tracking analyses. Hence, it might facilitate the work if either a differently coloured wall would be used, or if the wall could have been smaller (hence taking up less pixels on the videos).

High quality images of the genital papillae of the females will be taken at the beginning of the isolation period and inspected to see whether these truly are females (see below: floater males).

Floater males

In the previous round of experiments (2017), some of specimens identified prior to the experiment to be females turned out to have testes, even though they had a female colour pattern. These specimens were labelled 'floater males'.

Although the tracking data, and the behaviour data of these specimens have been collected and analysed we did not include them in the current version of the manuscript.

We should quantify how the behaviour of these individuals resembles that of females or males. Interestingly, one OV male displayed courtship behaviour, both towards an ON female as towards an ON floater male.

Brain parts of these specimens were collected, but not sequenced (yet)

We do not know whether these specimens were males from the start, or females that turned into males when they were isolated from (other) males. The latter process has been documented by aquarium enthusiasts. In order to find out whether the same will happen during the next round of experiments, high quality images of the genital papillae of the females will be taken at the beginning of the isolation period. At least one of the female OV specimens bought for the second experiment turned into a male.

RNA sequencing

Even though the new (both ON as OV) premating experiments mainly were set up to increase the number of replicates for the behavioural analyses, brain parts were dissected an stored in RNAlater. Hence, it is possible to use them for RNAseq.

This would cost roughly (see above):

- ON experiments: 8 replicates * 6 brain parts = 48 samples → €6,000
- OV experiments: 20 replicates * 6 brain parts = 120 samples → €18,000

As this would be an added value to the project, we will see whether there is still budget for this. For this, Erik suggested to have a look at a website that organises crowd funding for scientific projects: https://www.thebalancesmb.com/top-sites-for-crowdfunding-scientific-research-985238

Output strategy

We discussed whether we should try to combine the pre- and post-mating experiments in a single paper, or in two different manuscripts. Although we expressed our preference to not put too much data into a single manuscript, Maarten will make a pre-draft of the analyses by making a synopsis of figures and tables. Based on this, publication strategy will be determined.

Varia

Extra funds will be made available from BELSPO to allow for valorisation of the outcome of BRAIN projects. This would be for a budget of €20,000 (out of a total of 300,000 for 171 allegeable projects). The deadline of this would be the 26th of October.