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Project: Brain Sex Differences in *Oophaga sylvatica* Between Different Social States

Visit Narrative:

My research project focused on the genetics of behaviour, specifically on how gene expression changes between neuromodulators in different brain regions of the poison dart frog *Oophaga sylvatica* between social states and how this varies between the sexes. The poison dart frog used in my study, *O. sylvatica,* is an emerging model species and displays a range of ecologically significant behaviours. *O. sylvatica* is an obligate egg feeder, the female parent feeds the tadpoles trophic eggs which the tadpole eats exclusively. The females are choosy as to which males they mate with, as a result of this when males are together there is competition for females and fighting occurs. As such we considered 5 social states to look at the differences in brain gene expression: Male alone, Female alone, Male with Female, Female with Male, and Male with Male. My project tested this by using brains collected in the field from frogs exhibiting these behaviours, in total 8 brains were used for each of the 5 groups and each brain was dissected into 5 brain regions giving a total of 200 brain samples that were tested by qPCR for expression of 6 genes.

Differential expression of genes in the brain is central to understanding the basis of these behaviours and where these genes are expressed allows us to elucidate the molecular mechanisms by which these behaviours are controlled in the brain. My study tested the differential expression of 6 genes, 5 of which are implicated in the neurogenomics of social behaviours: Arginine Vasotocin, Mesotocin, Proopiomelanocortin (POMC), Galanin, and Luteinising Hormone Receptor (LHR). The 18s RNA gene was used as a reference as it is a constitutively expressed gene that differential levels of expression could be measured against.

The first step to conducting this study was to design primers for target genes and to research where in the frog brain these genes are expressed and their products found. Initially 12 genes were targeted. LHR was not one of the initial 12 but emerged as a target around halfway through the project as I learnt more about the genes implicated in these behaviours. Primers were designed from *O. sylvatica* sequenced DNA, however, for some target genes we did not have sequence so degenerate primers were designed for these from closely related species. Primers were tested by PCR and gel electrophoresis. Some primers designed were not specific enough and despite subsequent redesigning and testing were not workable in the time I had.

The second part of the project involved the dissection of *O. sylvatica* brains and extraction of RNA from the brain regions and conversion of this into cDNA. qPCR was ran on this cDNA using the primers designed. Data analysis is still on-going although preliminary data analysis does indicate differences between the sexes. LHR did not work so well, most likely because it is a receptor and is expressed in far smaller quantities than the other genes that we tested.

Possible improvements include testing further gene expression in the brain such as Androgen receptor. Histocytochemistry techniques could be used to identify the location of these proteins in the brain.