Flying under the influence

The humble fruit fly may hold the key to understanding individual differences in alcohol sensitivity and dependence. Here, Professor Robert Anholt outlines his work with Professor Trudy Mackay and Dr Tatiana Morozova on the genetic facet of alcohol pharmacology.

What stimulated your interest in using the *Drosophila melanogaster* model system to investigate the neurogenetic origins of alcohol abuse and dependence?

Behaviours are the ultimate expression of the nervous system, and *D. melanogaster* provides an ideal vehicle for the genetic dissection of behaviours. I am especially interested in deriving insights from the model that can be applied to humans, hence my interest in alcohol sensitivity.

Could you discuss the advantages and disadvantages of using an inebriometer to measure alcohol sensitivity in a *D. melanogaster* model?

Quantitative assessment of the effects of alcohol in people is often confounded by neuropsychiatric conditions and different measurements, such as drinking frequency, total amount of alcohol consumed per drinking session, age at first drink and withdrawal symptoms. The inebriometer, invented by Ken Weber, is advantageous since it allows precise, reproducible measurements of alcohol sensitivity as the mean elution time of flies from the column, both upon acute exposure and following the induction of tolerance. The limitation of the inebriometer is that it is low throughput and, thus, time consuming and labour-intensive if measurements of many different samples are required.

What is the relationship between alcohol sensitivity and the development of alcohol abuse and dependence disorders?

We must make a distinction between the acute sedative effects of alcohol, which result from the accumulation of alcohol in the circulation, and its long-term effects on the nervous system. Development of tolerance and continued drinking despite adverse effects can result in physiological dependence, or addiction, which is due to changes in the neural reward system, the dopaminergic mesolimbic projection in the brain. All aspects of the effects of alcohol, from sensitivity to acute inebriation to physiological dependence, which can result in severe withdrawal symptoms upon cessation of drinking, vary from individual to individual and depend on a predisposing underlying genetic architecture.

To identify candidate genes for human association studies, your lab employs both a single gene and a systems genetics approach. Could you elaborate on what these entail, and outline the strengths and weaknesses associated with each?

The propensity for alcohol consumption is a complex trait resulting from interactions between many genes and the environment. Therefore, we must understand how multiple genes act as ensembles, how interactions between them and the environment regulate the expression of other genes, and how this leads to the expression of their behaviour. This is the systems genetics approach, which is unbiased and looks at the whole picture. On the other hand, the candidate gene approach investigates the action of specific genes, which are often expected to be associated with the phenotype a priori. Although this is a more focused approach, both are useful and complementary.

Once the genomic elements that contribute to alcohol-related phenotypes have been identified, what is the next step towards translating these into human health benefits?

Understanding the genetic basis of alcohol-related phenotypes is relevant to personalised medicine. Individuals with a family history of alcohol dependence could be screened early for their propensity to develop addiction. Similarly, prognoses for rehabilitation could be informed by the genetic makeup that predisposes one to alcohol sensitivity.

How did you come to collaborate with Professor Trudy Mackay and Dr Tatiana Morozova, and how do your respective interests and areas of expertise benefit your research?

My background is in biochemistry and molecular neurobiology, and Trudy’s expertise lies in quantitative and statistical genetics. We met while horseback riding, got married and started working together. We have adjacent laboratories and have had synergistic collaborations for the last 25 years. And, yes, we still like horses.

We met Tanya Morozova through Elena Pasyukova, a collaborator from Moscow. Shortly after we received funding from the National Institutes of Health for our work on alcohol sensitivity, Tanya spent a year with us as a foreign exchange student, before joining our lab as a postdoctoral fellow. She has been the lead investigator on this project ever since.

By what means has your group distinguished itself in this research domain?

Human genetic studies on alcohol sensitivity have often focused exclusively on one-gene-at-a-time approaches, targeting known elements of the neural reward system. Although these studies have been valuable in their own right, they often miss the bigger picture by ‘looking for the lost key under the streetlight’, or identifying isolated players without their whole genomic context. Our work in *Drosophila* presents the most exhaustive description of alcohol sensitivity in any organism to date and demonstrates that alcohol-related phenotypes are emergent features arising from a complex underlying genetic architecture, including genes of unknown function, but implicated by the ‘guilt of association’ principle.
A true barfly: exploring alcohol sensitivity

How does one distinguish lager-lout from lightweight, or addict from abstainer? Research at North Carolina State University, USA, explores the effects of alcohol with the use of a novel comprehensive genetics approach.

Most civilisations produced alcohol before creating an alphabet, and the legal drug’s roots still run deep in societies and cultures worldwide, showing no obvious sign of recession. Scientists are, however, increasingly conscious of alcohol’s deleterious effects, and its ability to compromise judgement and reactions. In this time of designated drivers and responsible drinkers, a certain question is most pertinent: how can the consequences, both short and long term, of alcohol consumption for a given individual be predicted?

Seeking an answer to this question, Professor Robert Anholt and his collaborators, Professor Trudy Mackay and Dr Tatiana Morozova of North Carolina State University (NCSU), looked to one of the most thoroughly documented species in existence: Drosophila melanogaster, the common fruit fly.

The Drosophila Model
In human populations, the influences of nature and nurture are practically inextricable; neither genetic constitution nor environmental stimuli can be fully and effectively controlled. Worldwide, scientists are endeavouring to surmount this issue through the use of controlled experimentation, whereby only one variable is manipulated at any given time, so that this element alone can be confidently associated with any change in the observed outcome of an experiment.

Drosophila lends itself to scientific research for a host of reasons, but chief among them is the fact that a great number of genetically identical flies can be raised under controlled environmental conditions. In this way, researchers at NCSU are piecing together the genetic underpinnings of alcohol sensitivity, having controlled the interaction of genetic and environmental factors.

One might question the extent to which data derived from the common fruit fly may be generalised to the human organism. However, not only does Drosophila share with humans a considerable proportion of its genome, it also exhibits reminiscent behavioural and physiological responses to alcohol. Another important commonality of Drosophila and humans is the development of tolerance to alcohol in terms of chronic exposure, permitting the study of this phenomenon in the model organism. Furthermore, alcohol is ecologically relevant to the D. melanogaster species, being indicative of fruit fermentation and thus food, meaning alcohol processing-related genes feature prominently in the insect’s genome.

Alcohol sensitivity, and thus the development of tolerance, is subject to individual differences within and between human populations.
Alcoholism and Genetics

Alcohol dependence, characterised by physical withdrawal symptoms on cessation of alcohol consumption, typically follows the development of alcohol tolerance. Alcohol sensitivity, and thus the development of tolerance, is subject to individual differences within and between human populations. “Asian populations have an isoform of alcohol dehydrogenase, the enzyme that metabolises alcohol, which makes them more susceptible to inebriation and less likely to develop tolerance – a prerequisite for the development of dependence,” Anholt elucidates.

Anholt reports that predisposition to alcohol intoxication and addiction is an emergent property of the expression of multiple segregating genes, and their complex interactions with the environment. Consequently, there is no single gene that distinguishes the alcoholic, but rather genetic systems influenced by external factors such as stressors or trauma. Alcoholism is therefore said to be a ‘polygenic trait’.

Breakthrough publications emerging from Anholt’s group reveal that alcohol exposure flips a number of biochemical switches by altering the expression of a great many genes, concurrent with the development of tolerance. The team also identified that flies selectively bred for the trait of alcohol sensitivity possess a gene expression profile distinct from those bred for alcohol resistance, or unselected controls. Of these differentially expressed genes, 32 influenced alcohol sensitivity. Whilst a direct demonstration of causality in the relationship between gene expression and behaviour is a little way off, these findings constitute a truly remarkable contribution to the field of neurogenetics.

Found in Translation

Translational medicine has become quite the buzzword of late; researchers are encouraged to relate the findings of their basic science experiments to real-world issues, with the ultimate goal of enhancing human health and wellbeing.

It is therefore of relevance that 23 of the 32 alcohol sensitivity candidate genes identified by Anholt’s team have human equivalents, or ‘orthologs’, many of which are implicated in disease. Moreover, a subset of these genes are known to be differentially expressed in certain addiction-related regions of the alcoholic’s brain – circumstantial (though nonetheless convincing) evidence that these genes are functionally conserved in humans.

Further supporting the relevance of their model organism to human disease, Anholt and his colleagues found that the human counterpart of malic enzyme, associated with alcohol sensitivity in Drosophila, also contributes to variation in human alcohol consumption. Anholt expands on the significance of this finding: “Malic enzyme is a metabolic switch that facilitates fatty acid biosynthesis, resulting in fatty liver syndrome in heavy drinkers”.

The next step is a bench-to-bedside translation, potentially involving genetic screening to determine an individual’s sensitivity to alcohol or propensity for alcoholism. Such a procedure has the capacity to revolutionise the healthcare system’s approach to alcohol abuse, by introducing personalised preventive medicine.

Human Nature

For the future of his team’s work, Anholt envisions the integration of data from human and model organism studies. These data will be used to construct a framework through which gene interactions of relevance to alcoholism may be meaningfully delineated.

The group, however, has a number of more divergent plans. One such proposal pertains to alcohol exposure in utero, and the detrimental neurodevelopmental consequences of excessive drinking during pregnancy, termed foetal alcohol syndrome. Previous studies tell of the many variables that influence the syndrome’s presentation, including both maternal and foetal genes conferring susceptibility to alcohol-induced toxicity. Anholt and his colleagues will draw from, and build upon, these discoveries for their pioneering work in the future.