Travel grant application to support travel to France to take up an INRA/Agreenskills + fellowship (2015-2016)



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AGT 150**

Principal Investigator: **Dr Kate Howell**

Research Organisation: **The University of Melbourne**

Date: **9th Aug 2016**

Executive summary

This travel grant was used to support the trip of Kate Howell to take up a fellowship awarded by the European Union and INRA, in Montpellier France. The contribution by AGWA was to offset travel expenses not covered by my existing funding. My fellowship at INRA was to look at the populations of Drosophila and their yeast symbionts in French vineyards, with a particular focus on the pest species of Drosophila, spotted wing Drosophila (*Drosophila suzukii*). The work was carried out in two laboratories in Montpellier and surrounds; in UMR Sciences pour l'Oenologie (SPO) under the direction of Dr. Sylvie Dequin and at UMR Centre de Biologie et Gestion de Populations (CBPG) under the direction of Dr. Simon Fellous. My focus was understanding the microbial partners of Drosophila and I used both culture-dependent and culture-independent methods to characterise these communities. I tested how the flies are attracted to yeast substrates by correlating attractiveness with volatile production. The results are still forthcoming, but will contribute to the fundamental biological knowledge of this pest, by understanding the difference in microbial communities in different regional populations.

Deliverables

1. One peer-reviewed article on the microbial consortia of spotted wing drosophila by next generation sequencing
    a. This article is in preparation. The samples were collected during September and October in 3 distinct Languedoc-Roussillon vineyards at different stages of ripening around harvest. The samples were transferred to the laboratory and yeast and bacterial DNA was prepared using standard protocols. The samples were submitted to the AGAP sequencing facility (Montpellier, France) and will be run on the Illumina MiSeq next generation sequencing apparatus. The target date for this publication to be finalised and submitted is 31/12/2016.
2. Conferences and talks in Europe and visiting collaborators
    a. International Special Symposium on Yeasts, Perugia, Italy (September 2015). Talk entitled 'Yeast species associated with Drosophila in vineyard environments'. Abstract attached.
    b. 3rd AgreenSkills Annual Meeting 12-15 October 2015, Barcelona(Spain). The annual meeting brings together the fellows funded under the EU scheme, and both posters and talks are presented. Poster is attached.
    c. University of Bordeaux, France (November 2015). This seminar was given by invitation by Prof Isabelle Masneuf-Pomarede (University of Bordeaux, Bordeaux Sciences Agro) in Bordeaux. The seminar was one hour long and was well-attended by researchers at the institute.
    d. Local presentations at SPO and CBGP (regular group meetings and seminars)
    e. YeastCell summer school (Montpellier, 2016). The Marie Curie training school YeastCell has a node in Montpellier and the summer school was held there in June

2016. I attended and participated in three days of seminars by invited speakers and the PhD students in the program. My work done in collaboration with PhD candidate Mathias Eder was presented.

f. iGenolevures seminar (Utrecht, April 2016). I was invited to attend the iGenolevures symposium, which was held in one day at the CBS-KNAW Fungal Biodiversity Centre. I presented my work on yeasts associated with Drosophila at the pre-meeting conference, which was attended by the iGenolevures consortium (http://bim.igmors.u-psud.fr/wpigenolevures/). This consortium comprises researchers from CBS-KNAW Fungal Biodiversity Centre, CNRS, AgroParis-Tech, CRG, INRA and Universite Paris Sud.

g. SWD research retreat (June 2016) with Dr Patricia Gibert (CNRS, Lyon), Dr Simon Fellous (INRA, Baillarguet), Dr Allan Debelle (University of Sussex, UK) and local lab members from Montpellier.

3. Extension and report

a. Article in Wine and Viticulture Journal on the problem of SWD in vineyards, likelihood of an Australian invasion and implications for the Australian wine industry. I have discussed the scope and dates with the editor Sonja Logan, and have set a date for publication before the end of 2016. The manuscript has been prepared and I am corresponding with state government agencies about reporting sightings in Australia.

Abstract for a talk presented at the 32nd International Specialised Symposium of Yeasts, Perugia Italy, September 2015.

Session 2A: Yeasts in food biotechnology: biodiversity and ecology in foods and beverages

Yeast species associated with *Drosophila* in vineyard ecosystems

Samuel S.T.H Lam[1] and Kate S. Howell[1,*]

[1]Faculty of Veterinary and Agricultural Sciences, University of Melbourne Parkville Victoria 3010 Australia

*khowell@unimelb.edu.au

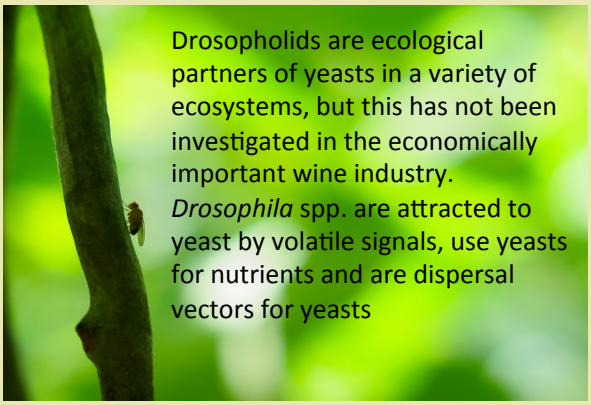
The activity of yeasts in wine fermentations directly contributes to wine quality, but the source and movement of these yeasts in vineyard and winery environments has not been resolved. This study investigates the yeast species associated with a insect vector to help understand yeast dispersal and persistence. Drosophila are commonly found in vineyards and *Drosophila* and yeasts have a known mutualistic relationship in other ecosystems. Drosophilids were collected from vineyards, marc piles and wineries during the grape harvest. Captured flies were identified morphologically to and their associated yeasts were identified. Of the 296 *Drosophila* flies captured in this study the species identified were *Drosophila melanogaster, Drosophila simulans, Drosophila hydei,* and *Scaptodrosophila lativittata*. These flies were associated with the yeasts *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Torulaspora delbrueckii* and *Hanseniaspora valbyensis*. The diversity of yeasts and *Drosophila* species differed between collection locations (vineyard and marc; R=0.588 for *Drosophila* and R= 0.644 for yeasts). Surprisingly, the primary wine fermentation yeast, *Saccharomyces cerevisiae* was not isolated in this study. *Drosophila* flies are preferentially associated with different species of yeasts in the vineyard and winery environment and this association may help movement and dispersal of yeast species in the vineyard and winery ecosystem.

KEYWORDS: Drosophila; vineyard; yeast diversity; biogeography; wine

Drosophila-associated yeasts in winegrowing environments

Kate Howell

INRA, UMR1083 Sciences pour l'Oenologie, Montpellier 34060 France
Faculty of Veterinary and Agricultural Sciences, University of Melbourne Parkville 3010 Australia
kate.howell@supagro.inra.fr; khowell@unimelb.edu.au



Drosopholids are ecological partners of yeasts in a variety of ecosystems, but this has not been investigated in the economically important wine industry. *Drosophila* spp. are attracted to yeast by volatile signals, use yeasts for nutrients and are dispersal vectors for yeasts



The main wine yeast, *Saccharomyces cerevisiae,* is found in high numbers in wineries, but is difficult to find in the vineyard

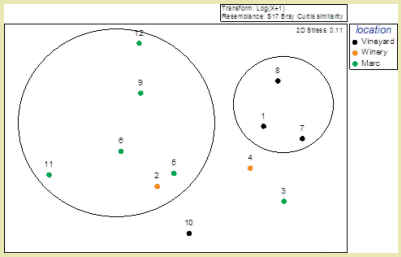
Understanding the yeast communities in winemaking is important to predict and control the quality of the resultant wine. The geographic distribution and dispersal of yeasts by *Drosophila* spp. may be important for the incidence of yeasts, and thus the particular aromatic signatures of wine grown in a particular area (*terroir*).

This project:

1. Culture-independent assessment of yeasts present on Drosopholids (next-generation sequencing of yeast communities)
2. Extensive sampling in vineyards and wineries in the Languedoc-Roussillon region
3. Chemical profiling of yeast volatiles and their attractiveness to *Drosophila*



The recently introduced pest, spotted wing Drosophila (*D. suzukii*) is a particular focus of this project (image eppo.int). Unlike other Drosophilia, this fly can injure intact ripe fruit and causes significant economic losses.



NMDS figure showing the clustering of yeasts species found at different environmental collection points in winegrowing environments. Yeasts (and flies) found at particular habitats are different to one another.

Lam & Howell (2015) FEMS Micro Letters (accepted)

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