

DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: Viral Pathogens of *Drosophila suzukii*

Travel Dates: 11th – 25th June 2016

Location: Tokyo, Japan

Group Members: Dr Madoka Nakai, Dr Darren Obbard

Aims: To sample the viruses of *Drosophila suzukii* in its native range.

Outcome (not less than 300 words):-

Expedition Report:

Viral Pathogens Suitable for the Control of *Drosophila suzukii*:

An expedition to sample virus diversity with the pest's native range.

Nathan Medd - *The University of Edinburgh*



THE UNIVERSITY
of EDINBURGH



東京農工大学

Tokyo University of Agriculture and Technology

Project Introduction

Drosophila suzukii (Matsumura) is an invasive dipteran pest of soft fruits. Its recent invasion of the fruit growing regions of North America and Europe and the damage it has caused there have driven interest in finding new control solutions. Conventional chemical control methods have many environmental drawbacks and are difficult to implement for this pest, consequently the development of an alternative, ecologically benign biopesticide would be extremely beneficial for fruit growers, consumers and rural biodiversity alike.

This study seeks to characterise the viral diversity of *D. suzukii* with the aim of identifying a pathogen suitable for the control of this pest in UK fruit crops. Viruses offer an interesting source for a microbial biological control agent as they potentially have high host specificity and low environmental persistence (Hunter-Fujita et al., 1998). During this study I do, however, seek to answer some more fundamental questions about the nature of viruses and the role they play in shaping their hosts.

Expedition Aims

The aim of this expedition was to investigate the viral diversity infecting *Drosophila suzukii* in its native range: not only to maximise chances of discovering potential control agents but also to elucidate the patterns of virus prevalence and abundance in a recently invasive species.

During this expedition my primary aim was to catch as many wild *Drosophila suzukii* as possible in order to maximise the chances of discovering rare viruses present in a small percentage of the fly population. Secondary to this aim, other species of drosophila, especially species which are also found in the UK, were also sought in appreciable numbers. These other species may be infected by closely or distantly related viruses but the exact nature of host shifting by viruses in the wild is still poorly understood.

Further to the technical aims of this expedition, a collaborative relationship between the University of Edinburgh and the Tokyo University of Agriculture and Technology (TUAT) was a desired outcome of this visit.

Locations and Methods

Techniques for sampling wild flies included sweep netting, bait trapping and collection of infected fruits. Traps were baited with cotton wool soaked in an attractant vinegar-based liquid. Larvae were sampled after dissecting fruits using forceps and scalpel.

Japan, thought to be within the native range of *D. suzukii*, and was an excellent destination to investigate these aims as much scientific work here is focused on its control. Japan has dealt with this fly as an agricultural pest since 1916 when damage was first reported. Different prefectures within Honshu (see figure 1) have varying amounts of soft fruit production and the local government scientists work relatively independently of those in other prefectures.



Figure 1: Map of Japan, showing prefectures.

Tokyo

The University of Technology and Agriculture, the base for this expedition is located in Fuchu within the city of Tokyo. It has a relatively small plot of cultivated land used to teach agricultural techniques to students. This includes plots of blueberry (*Vaccinium* spp.) bushes, of a number of different varieties, which suffer with some damage by *D. suzukii* (Figure 2). Bait traps were placed in these areas, as well as a nearby mulberry (*Morus* sp.) tree, and provided a steady catch of fruit flies through the duration of the trip.

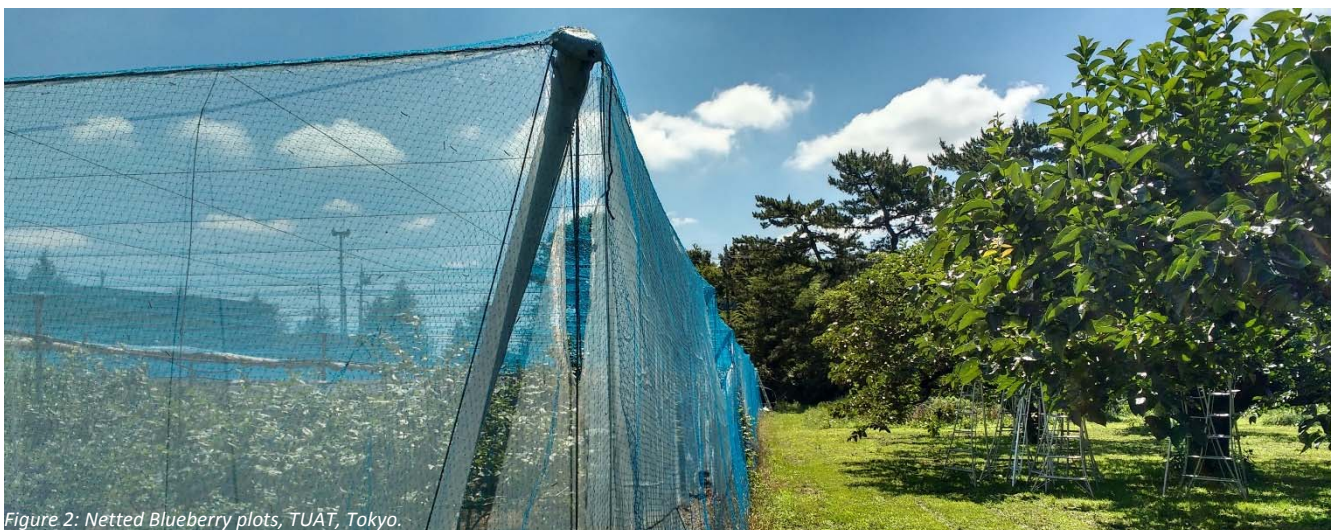
Additional trapping locations within Tokyo were also exploited: Naganuma Park and Tama Hills, to the west of Fuchu, boasted a large number of wild cherry (*Prunus* subg. *padus*) and mulberry trees, potential host species of *D. suzukii*. A public park and a university run climate research station respectively, both were relatively unsuccessful sampling locations, yielding just a handful of flies each.

Due to a warmer than average spring the fruit trees in this particular area (all locations within Tokyo were at approximately 35.5° latitude and between 50 & 150 MASL) were somewhat advanced compared to previous years. In fact main fruit fall had been approximately two weeks earlier. This negatively affected fruit fly catch and although some *D. suzukii* were caught in these areas, numbers were disappointing.

During this trip The Tokyo University of Agriculture and Technology insect pathology laboratories were used as the primary location for sample storage and fly identification.

Yamagata

In the second week of the expedition we travelled northwards to the cherry growing region of Yamagata. Being considerably further north than Tokyo the fruiting period of *Prunus* spp. here was still ongoing. Yamagata has a large area of cultivated sweet cherry and is famed for its production of cherries specifically. We visited the Yamagata prefecture soft fruit research facility in Sagae, Yamagata. At this research station we unable to catch a large number of fruit flies however researchers working at the facility were able to provide some flies caught recently emerging from damaged fruit. The reason for this low fly abundance was thought to be the effective use of chemical pest controls at the facility.



Fukushima

The district of Fukushima, despite recent decline, was a fertile and productive region of Japan. Associated heavily with soft fruit production, Fukushima represented another potentially good sampling location for *Drosophila*. We visited researchers at the 'Agriculture Synthesis Centre Fruit Tree Research Institute' and were able to catch a number of *Drosophila* species in the institute's fruit growing plots. At no point during our visit to the Fukushima prefecture did we come near the official exclusion zones in the east of the region.

Yamanashi

This prefecture is also known for its production of fruit: primarily table grapes and peaches. More mountainous than other areas visited during this expedition, its increased altitude meant certain wild cherry trees were still producing fruit alongside commercial harvested varieties. This combination of fruiting host plants yielded the highest numbers of *D. suzukii* of any location visited.

Preliminary Results

In total 278 individual *D. suzukii* were caught during this trip along with 352 other individual *Drosophila* of four other species. Flies were transported by air, dead, whole and in clean 1.5ml Eppendorf tubes. On return to Edinburgh flies were macerated in sterile Ringers solution and a portion of this homogenate taken forward to Phenol-Chloroform RNA extraction. Reverse transcription reactions allowed the creation of cDNA for all samples and PCR's, using a selection of previously designed primers, are being used to screen all samples for the presence of known *D. suzukii* viruses. Preliminary results suggest that flies in Japan are not infected with very many of the viruses which infect flies in the UK and Europe. Of the 25 viruses so far screened for in *D. suzukii*, only two viruses have been positively identified in these samples. This is an interesting result as it either represents a lower diversity of viruses in the native range of this pest or it suggests that the primers, designed for UK viruses, do not work on the viruses infecting *D. suzukii* in its native range.

To further investigate the viruses present in these samples we will be including a pooled sample of these Japanese flies in a round of metatranscriptomic sequencing alongside further British samples in the autumn. This will allow the discovery of viruses not yet described in previous European samples of *D. suzukii*.

In addition to the fly samples that will continue to yield much interesting information, I also gathered a great deal of useful information about the way in which *D. suzukii* is dealt with as a pest in Japan. Differences in the



Figure 3: Dr Takabe of the Yamagata prefecture fruit research station inspecting for damaged cherries.

chemical control products available seems to be the primary explanation for why this fly is not exceptionally damaging to fruit crops here. Organophosphates, banned in Europe but available in Japan, are a very useful tool for growers wanting to control pest numbers. Chemical advantages are not, however, the only difference in control practice: cultural control also plays a part. Japanese fruit growers are accustomed to the practice of maintaining high crop hygiene standards. Removing dropped fruit and disposing of it correctly is seen as essential to avoid the build-up of fruit flies.

Budget

The total expenditure for this expedition came in within budget with some surplus to be returned to the Davis expedition fund (see table 1). Full and detailed record of expenses and receipts available on request.

Element	Predicted Cost	Actual Cost
Return Air Travel	£800	£806
Internal Travel and Transfers	£200	£348
Accommodation (2 weeks)	£850	£640
Living expenses (2 weeks)	£300	£342
Lab reagents and consumables	£300	£96
Travel insurance	£50	£0
Visas and Collection permits	£0	£0
Total	£2500	£2232
Remainder to be returned to fund		£278

Acknowledgements

I would like to thank: Madoka Nakai and all the staff students at the TUAT for their considerable hospitality, Dr Takabe and Dr Suzuki at Yamagata research station, Mitsuru Abe and Kouki Yoshida at Fukushima prefecture research station and Mr Uchida and Kunitomo at Yamanashi prefecture.

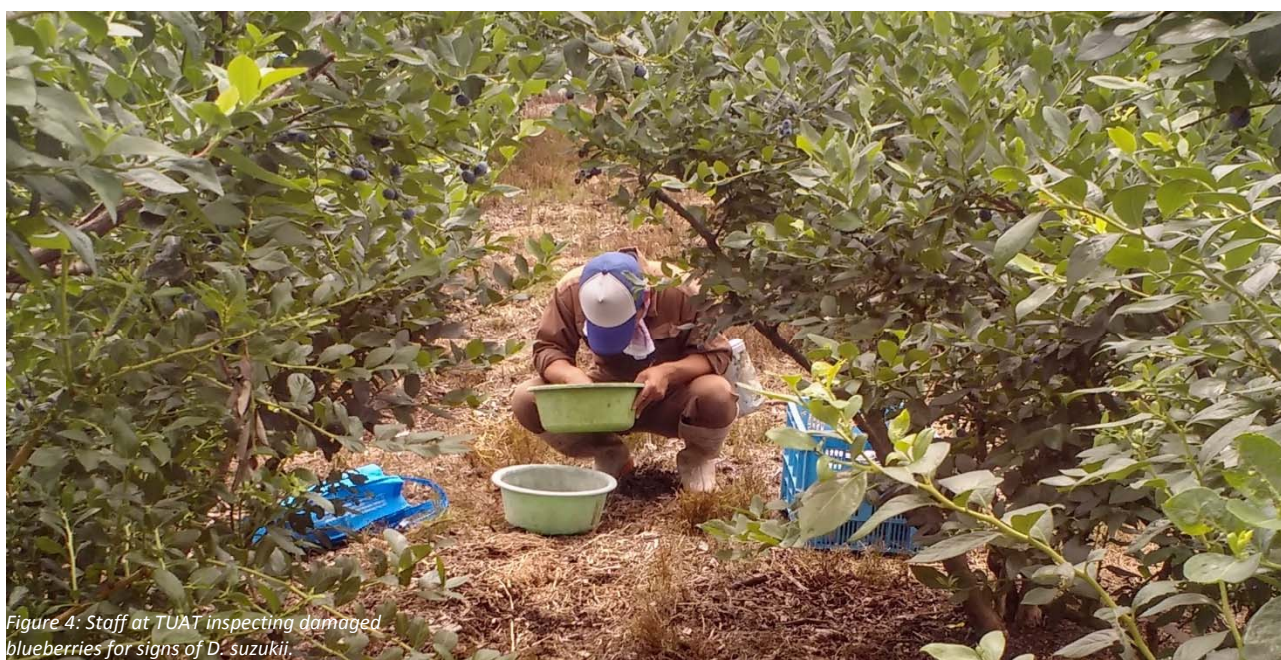


Figure 4: Staff at TUAT inspecting damaged blueberries for signs of *D. suzukii*.