DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: The evolution of Paternal Genome Elimination in the pest species, *Pseudococcus viburni*

Travel Dates: April-May 2017

Location: California, USA

Group Members: Stevie Anne Bain, Isabelle Vea

Aims: To collect mealybugs from California populations known to possess B chromosomes for immunostaining and establishment of laboratory cultures in Edinburgh

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The evolution of Paternal Genome Elimination in the pest species, *Pseudococcus viburni*

Pseudococcus viburni infestation at Central Coast vineyard.

(California Department of Food and Agriculture)

My PhD research focuses on paternal genome elimination (PGE) an unusual type of reproduction found among several insect species, including mealybugs (e.g. *Planococcus citri* and *Pseudococcus viburni*). In sons, the paternally inherited set of chromosomes is silenced and then eliminated from the germline. Since males can only pass on their genetic information through daughters, it is in their interest to either prevent elimination from happening or to female-bias their offspring's sex ratio. However, females have an evolutionary advantage through sons, as sons can only pass on their

maternally inherited chromosomes to any offspring they sire. Thus, a sexual conflict between both parents occurs.

Although PGE is found in thousands of species of insects, including many economically important pests and parasites, we understand very little about the mechanism responsible. It is, for example, unclear how the parental origin of chromosomes is recognized, how this leads to their difference in behaviour and why this differs between males and females. Previous studies suggest that it is regulated by DNA methylation and histone modifications, mechanisms used across the tree of life for the regulation of gene expression. However, the molecular details remain poorly understood. My research focuses on understanding the mechanism by which PGE acts in the citrus mealybug, *Planococcus citri*. Specifically, I investigate patterns of DNA methylation on maternally and paternally inherited chromosomes. However, one of the key challenges in studying how PGE works is that it happens in every single citrus mealybug male in the population. As a result, it is not possible to compare males that do and males that do not eliminate their father's genes to assess how they differ. I am therefore keen to shift my focus to a different mealybug species: the obscure mealybug, *Pseudococcus viburni*. In some, but not all individuals of this species, males carry a chromosome that can "cheat" PGE to avoid elimination even when it is inherited from the father. Therefore, comparing males with and without this chromosome could generate new insights, beyond what my current study organism has to offer. I therefore request funding for a trip to California to sample the populations where this "extra" chromosome was first discovered.

Pseudococcus viburni, like *Planococcus citri,* possess 10 chromosomes as standard: 5 inherited from the mother and 5 inherited from the father. In females, all 10 of these chromosomes are in a euchromatic state, a state in which genes are transcriptionally active. In males, the chromosome condition is significantly different: The 5 chromosomes inherited from their father are silenced and then subsequently eliminated from their germline. *P. viburni*, however, possess an extra "B" chromosome that is transmitted at a higher rate than would be expected according to Mendelian inheritance (Jones and Rees, 1982). These chromosomes are in a state in which genes are not transcriptionally active (like paternally inherited chromosomes). However, during sperm production, B chromosomes change their chromosome state to resemble maternally inherited chromosomes (Nur *et al.,* 1988). This system, therefore, provides a rare chance to understand how the parental origin of a chromosome is recognized and how this this determines its fate during meiosis.

Although *P.viburni* is a cosmopolitan species, B chromosomes have only been found in Californian populations (Nur *et al.,* 1987). Therefore, it is key to investigate these populations, which are found on the Pacific Yew, *Taxus brevifolia*, a conifer native to the Pacific North West of North America and also in vineyards throughout the Central and North Coasts of California.

Expedition aims

I requested funding to collect *Pseucococcus viburni* samples from their natural habitat in California. Specifically, I revisited those populations in which the B chromosome was first discovered (Nur *et al.,* 1987). Other mealybug species that our research group studies, *Planococcus citri* and *Planococcus ficus*, were also collected to broaden the genetic diversity of our laboratory cultures.

Methods

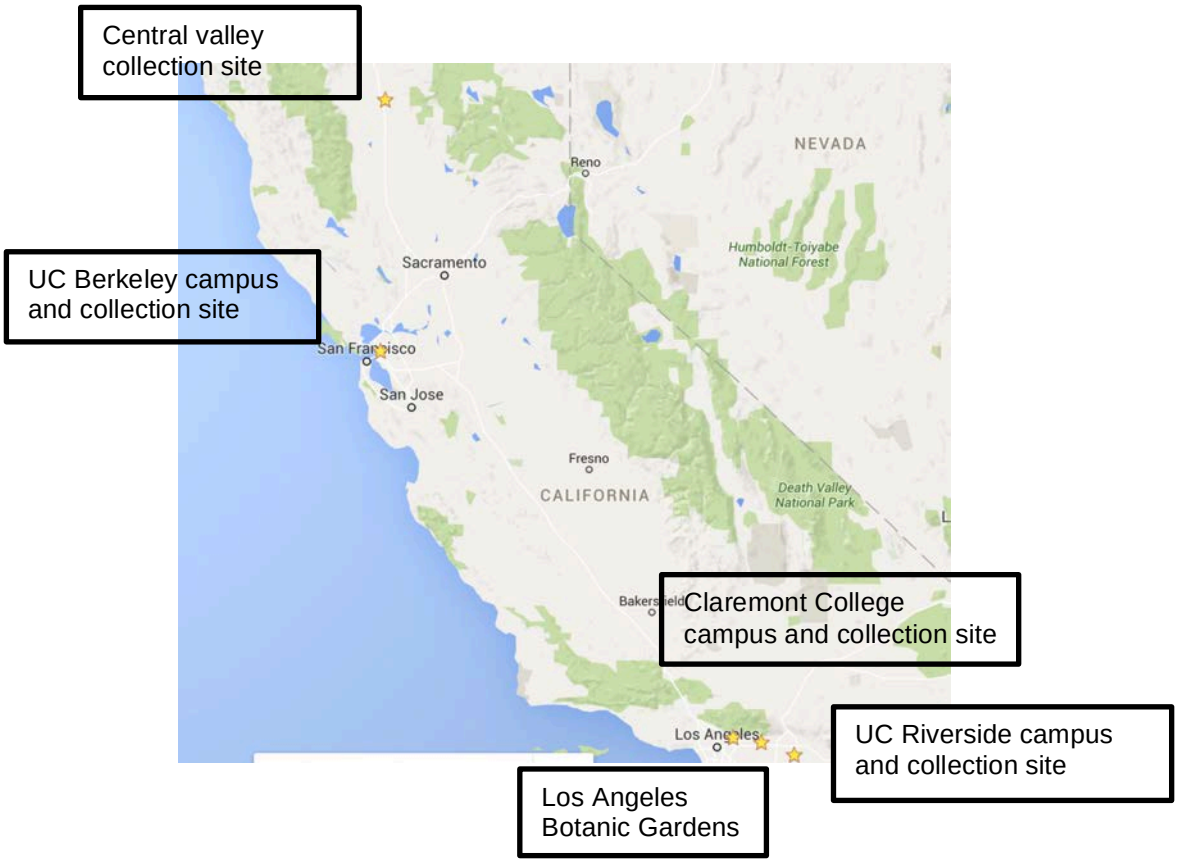
I collected live adult mealybugs, which are found on host plants (*Taxus brevifolia* and grape vine plants, *Vitis californica*) and also egg masses. The adult females are relatively sessile and can be easily collected from host plants using a small paintbrush. The winged males are much smaller than females and only live as adults for 2-3 days. I collected insects from each of the sites. The generation time of *P.viburni* is ~30 days, with adult males developing at, on average, day 20. Therefore, a 4-week trip allowed time for the collection of the mealybugs and examination of all life stages.

A number of the females collected from each of the sites were used to establish genetically inbred laboratory lines, allowing further work on this pest species to be carried out. We possess all necessary permits.

Female *Pseudococcus viburni*

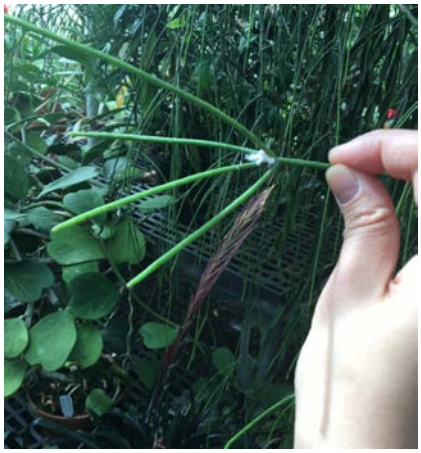
Laboratory cultures are kept in specially made boxes (with a mite proof mesh, allowing air flow) on sprouting potatoes at 25°C (60% humidity). Mealybugs are easily kept within the laboratory environment and reproduce successfully in these conditions.

During my time in California, I worked with Dr Isabelle Vea, a Post-Doctoral researcher and skilled entomologist. Her expertise ensured that species were correctly identified. Together we traveled around California (see map) to collect the species needed to carry out our research. These collection sites were located nearby research institutes willing to share the laboratory space and equipment necessary to carry out staining of chromosomes and also microscopy work: UC Berkeley, UC Riverside and the Claremont Colleges, CA.



Professor Patrick Ferree (Claremont Colleges) also hosted us for some time during our fieldwork. His work focuses on understanding the mechanisms by which selfish genetic elements are able to manipulate insect reproduction. In particular, he has used cytogenetic techniques to show that a B chromosome in the jewel wasp *Nasonia* induces histone modifications leading to the elimination of the paternal genome (Swim *et al.,* 2012 & Ferree personal communication). Remarkably this pathway appears to shows strong similarities to that involved in PGE in mealybugs. Visiting the Ferree lab allowed me to rely upon his expertise and to apply the methodology developed for *Nasonia* to the mealybugs. Since not all *P. viburni* males possess these “extra” B chromosomes, we can directly compare males that possess B chromosomes to those that do not. Chromosome staining of these organisms will show how the histone modifications of B chromosomes differ from those of autosomes, and how this may be affecting their condition. Identifying the histone modifications involved in the changes of B chromosome condition will provide insight into how the paternally inherited chromosomes of males can become silenced during paternal genome elimination in *P. citri.*

Collecting mealybugs in a vineyard in Northern California.



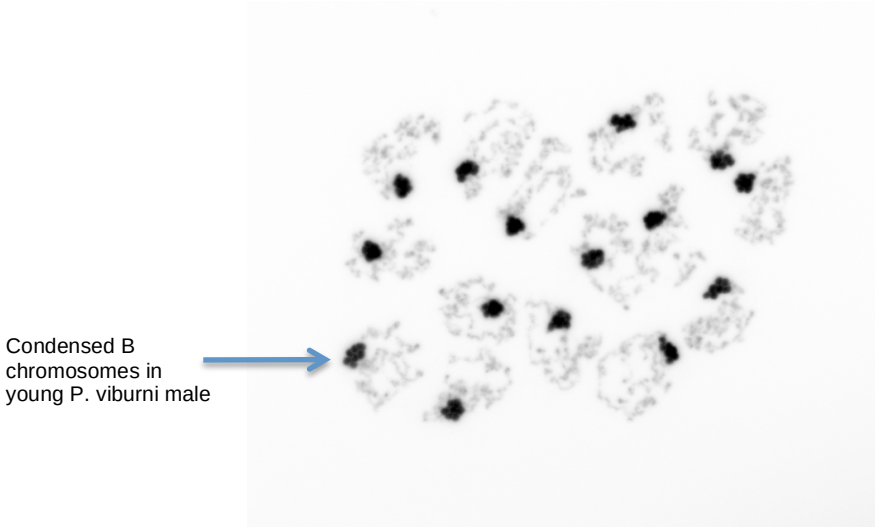
An egg mass deposited by a *Pseudococcus viburni female* on a plant in a glasshouse at UC Davis.

Outcomes

Overall, the aim of the expedition was achieved. We collected mealybug species, including *Pseudococcus viburni*, from a number of vineyards and glasshouses in locations throughout California. These species were transported back to our laboratory in Edinburgh. We then carried out microsatellite analysis to identify different species.

We confidently identified 3 different species using molecular techniques and went on to generate inbred cultures for use in our experimental studies. Once the populations of these laboratory cultures were well established, we carried out karyotyping to identify populations with 'extra' chromosomes, known as B chromosomes.

We have identified several populations of *P. viburni* possessing these supernumerary B chromosomes (see image below). Currently, we are preparing to sequence these individuals to generate the B chromosome DNA sequences and identify whether or not these chromosomes carry genes that may allow them to escape the process of paternal genome elimination.



The above image shows the chromosomes of a 3rd instar *Pseudococcus viburni*. Supernumerary B chromosomes are present.

This project has provided me with invaluable fieldwork experience, which would otherwise be unavailable to me, and also allowed me to expand the scope of my PhD research by working on a different species of scale insect. Also, I work in an evolutionary biology department and neither my supervisor, nor anyone else in the department, is able to teach me the cytogenetic techniques required to investigate B chromosome epigenetics. Therefore, this collaboration with researchers in UC Berkeley, UC Riverside and Claremont College has made a significant contribution to my

scientific education. We have also established connections with vineyard and glasshouse managers throughout California who can potentially supply us with species of mealybug and population data.