DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	Papayas, ants, and pollination Florivory
Travel Dates:	15 September 2018 – 25 October
Location:	Chamela, Jalisco & Zihuateutla, Puebla. MEXICO
Group Members:	Nora Villamil Buenrostro
Aims:	To assess the effect of ant-plant interactions on the host plant pollination in an applied agroecosystem: papayas. To collect data on florivory and herbivory levels on a Mexican population.

2017 EXPEDITION REPORT
Papayas, ants and pollination
Florivory

Ecological costs of myrmecophily in an agroecosystem
Florivory meta-analysis

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I want to thank the Davis Trust for the £2995 grant awarded to me in 2017. The fieldwork expedition to Mexico was originally planned for 2017 and aimed to investigate ant-plant interactions in *Acacia (=Vachellia) hindsii* in Chamela, Jalisco, Mexico. However due to major unforeseen circumstances explained previously (please see attached to this report the extension request letter sent previously) I had to delay the expedition until 2018. The 2018 expedition ended up investigating different questions than those stated in the 2017 proposal, but adhered to the ant-plant topic at Chamela, Jalisco, Mexico.

In 2018, I explored the consequences of plant-ant interactions in an agricultural system: papaya (*Carica papaya*, Caricaceae). Additionally, I conducted two other projects (Florivory and *Turnera*) detailed below. I am very grateful for the funds that the Davis Trust generously provided me, as they allowed me to start three new collaborations on tropical ecology between the University of Edinburgh, Mexican and Brazilian research institutions.

Please find below a summary of the projects conducted, along with some of our key findings or observations (see section: Projects, activities and findings). Details of the outcomes I have achieved thanks to Davis Trust Funds are also detailed further down (see section: Outcomes achieved with Davis Trust Funds). All the funds granted were used for this fieldwork expedition and expenses details are provided below in Table 1. The work reported below could not have been achieved without the help of a brilliant field assistant who volunteered for long hours: Salomón Vergara, and some Mexican collaborators: Ana María Flores, Prof. Ek del Val, and Xóchitl Damián.

Figure 1. Map of the locations where fieldwork for the different research projects funded by the Davis Trust was conducted. The effects of ant patrolling on the pollination biology and yield of papayas was explored in Chamela, Jalisco. Data on levels of florivory and herbivory in a rainforest were collected in Zihuateutla, Puebla. Samples from an experiment on the effect of pollen origin on fruit abortion rates in *Turnera velutina* were processed in Mexico City.

Table 1. Detailed costs and providers', and contributions towards the 2017/2018 fieldwork expedition supported by the Davis Trust.

	Item	Cost (£)	Provider	Contributions (£)
TRAVEL	**UK-Mexico return flight**	1572	Davis Trust	1572
	Transport to Chamela (buses + taxis)	100	Davis Trust	100
	Transport to Puebla (petrol + road fares)	100	Davis Trust	100
	Car expenses and insurance	250	Davis Trust	250
			Travel Sub-Total	**2022**
SUBSISTANCE	**Food and expenses at Chamela**	100	Davis Trust	100
	Accommodation and food at Puebla	300	Davis Trust	300
	Additional expenses Puebla	80	Davis Trust	80
	Food and expenses Mexico City	250	Davis Trust	250
			Subsistence Sub-Total	**730**
SUPPLIES	**Disposables** (Stationary, tags, jars, bags, ant excluding resin)	80	Davis Trust	80
	Botanical press	50	Davis Trust	50
	Other equipment (GPS with altimeter)	263	Davis Trust Nora Villamil	113 150
			Supplies Sub-Total	**393**
			TOTAL FIELDWORK COSTS	**3145**
			AWARD PROVIDED	**2995**

Projects, activities, and findings

Papayas, ants and yield

Ant-plants are defined as plant species that recruit ants to defend themselves against herbivores, in exchange for food or housing rewards for the ants (Rico-Gray and Oliveira 2007). These are phylogenetically diverse group of plants comprising >4000 angiosperms (Keeler 2014), out of which >80% are likely to require pollinators to set seeds (Ollerton et al. 2011, Keeler 2014). However, the effect of ant patrolling on the pollination biology of ant-plants has been scarcely studied in general, and even less so, in agroecosystems.

Papayas (*Carica*) attract ants as natural enemies of herbivores, but and also rely heavily on pollinators to set seeds, especially given that many papaya species are dioecious[1]. Mesoamerica is likely to be the centre of origin and domestication of this crop, with many native species and cultivated varieties (Chávez-Pesqueira and Núñez-Farfán 2017). Additionally, papaya is a commercial crop, in fact the third most cultivated tropical crop worldwide (Evans and Ballen 2012). Furthermore, Mexico is the fifth worldwide producer of papaya and the main exporter of papayas with exports worth >93 million dollars per year (SAGARPA).

Apart from being a commercially important crop, papaya is also a biologically interesting system to investigate tropical ant-plant-pollinator interactions. Previous findings on the effect of natural herbivores on papaya yield showed a direct link between the presence of ants and the yield of papaya orchards, with this link being unrelated to herbivory levels (Flores *et al*. in prep.). This opened up interesting research avenues to unravel other ant-plant interactions and their effect on plant fitness/yield. In collaboration with Ana María Flores and Ek del Val from the Institute of Ecosystem and Sustainability Research at UNAM, Morelia, Mexico, we investigated the following questions:

1. Does ant patrolling differ between sexes in papaya (*Carica papaya* L. c.v. Maradol) (female, male and hermaphrodite)?
2. Does ant patrolling affect pollen transfer in papaya?
3. What is the role of ants on the pollination biology and yield of papaya?

<u>Preliminary key findings:</u>

1. Preliminary analyses showed that the abundance of ants patrolling plants differed across sexes. Higher numbers of ants were found on male plants, followed by hermaphrodites, and finally female plants.
2. Stigmas collected from control (with ants patrolling) or experimental (ants excluded) female papaya plants are being processed to count the number of pollen grains transferred, to assess the effect of ants on pollen transfer to this dioecious species. Since female plants have no pollen-bearing flowers, all pollen grains found on these stigmas must have been transferred from neighbouring male plants.
3. Ants may be directly involved in papaya pollination. Ants were found inside mature female floral buds, which were previously reported as cleistogamous[2]. Preliminary evidence suggests these flowers may not actually be self-fertilised as floral buds, but actually be pollinated by visiting ants. Ant samples are being processed, looking for viable pollen grains on ants. If ants ere successful papaya pollinators, this would explain the direct link between ant presence and yield observed by Flores *et al.* (unpubl. data).

[1] Dioecious: plant bearing unisexual male and female flowers on different individual.
[2] Cleistogamous: with self-fertilisation occurring within the unopened flower.

Florivory

During the symposium "Insect-Plant interactions: patterns and processes in a changing world" at the ATBC Meeting 2017 held in Mérida, México the attendants- myself included- discussed that herbivory levels reported in the literature might be underestimated with respect to levels observed in the field, a pattern especially strong for tropical species. We also noticed that florivory has been widely overlooked with no study so far synthesising world levels of damage to tropical or temperate flowers.

In order to address and evaluate such discrepancy, we designed a florivory and herbivory sampling protocol and set up in a mission to collect data worldwide as an inclusive and collaborative effort lead by Prof. Cornelissen from the Federal University of São João del-Rei, Brazil. However, this project is unfunded as a whole. The Davis Trust grant allowed me to sample florivory and herbivory levels in a rainforest within the Sierra Madre Oriental, a mountain range in the northeast of Mexico, and so contribute with another location to reassess the global levels of florivory and herbivory, paying close attention to tropical ecosystems.

Preliminary key findings:

Levels of leaf and flower damage differed among and within species. However, these data and samples are still being processed and we hope to have global patterns soon, comparing samples taken at different locations.

Turnera velutina

Finally, with this funding I was also able to process data from an experiment on *Turnera velutina* (Passifloraceae) exploring the effect of pollen origin (selfing or outcrossing) on fruit abortion. Previous findings showed that ant patrolling affect the host plant (*Turnera velutina*) pollination biology and mating system (Villamil unpubl. data). Ant patrolling significantly decreased visit duration, pollen load on stigmas and male fitness, and increased outcrossing rates. Furthermore, plants with reduced ant patrolling resulted in a significant 12% increase in the rates of fruit abortion.

I hypothesised that fruit abortion may be linked to pollen origin, via selective plant abortion or inbreeding depression, leading to an increase proportion of selfed fruits in plants with low levels of ant patrolling and high selfing rates. If such hypothesis was true, then this would be the first evidence of ant patrolling affecting plant pollination biology, plant mating system and plant fitness.

Along with Xóchitl Damián from the LIPA lab (Institute of Ecology, UNAM) we conducted an experiment to test whether fruit abortion is linked to pollen origin and selfing rates. In 60 *T. velutina* plants, we self-pollinated half of the flowers and outcrossed the remaining flowers, and assessed fruit abortion and seed number on fruits developed from both treatments.

Preliminary key findings:

Our preliminary counts suggest that selfing is linked to higher fruit abortion rates, although further statistical analyses are required. These results will be included in a peer-reviewed paper on the ecological costs of ants for the host plant pollination in *T. velutina*.

Outcomes achieved with Davis Trust Funds

I have summarised (Table 2) all the products and outcomes achieved with the grants the Davis Trust has kindly awarded me. Thanks to the Davis Trust grant awarded in 2017 and used in the 2018 expedition I have or will produce the following outcomes:

- A peer-reviewed paper on the on the effects of ants on the pollination and yield of papaya, a crop of major economic importance in Mexico (in prep.).
- A peer-reviewed meta-analyses on worldwide florivory (in prep.).
- A peer-reviewed paper on fruit abortion rates linked to pollen origin (selfing *vs.* outcrossing) in *Turnera velutina*.

Table 2. Products and outcomes achieved with the grants awarded by the Davis Trust Funds to myself, Nora Villamil Buenrostro.

Year awarded	Product	Type	Details		Status
			Title	Publisher	
2016	Publication	Peer-reviewed	Ant-Pollinator conflict results in pollinator deterrence but no nectar trade-offs	Frontiers in Plant Sciences	Published 2018
2016	Publication	Science communication	Published in Spanish: (Coastal sand dunes: a coveted, overlook and threatened ecosystem) Dunas costeras: un ecosistema codiciado, soslayado y amenazado	Magazine from the National University (UNAM)	Published 2018
2016	Publication	Peer-reviewed	Testing the Distraction Hypothesis: do extrafloral nectaries reduce ant-pollinator conflict?	Journal of Ecology	Accepted (in press) 2019
2016	Conference	Talk	"Juggling ants and bees: mutualist management in ant-plants"	Association for Tropical Biology and Conservation (ATBC)	July 2017
2016	Conference	Talk	"How costly are the mercenaries? Ecological costs of myrmecophily"	Mexican Ecological Society	August 2017
2016	Conference	Talk	"Ant-Pollinator conflicts in ant-plants" First place, Best long talk	Scottish Tropical Ecology and Biology (STEB)	March 2018
2016	Dissertation	PhD	Ant-pollinator interactions in *Turnera velutina*: ecological costs and evolutionary consequences for the ant-plant pollination	University of Edinburgh	Awarded 2019
2016	Publication	Peer-reviewed	Ecological costs of ant protection for the host plant pollination.		In prep.
2017	Publication	Peer-reviewed	Does selfing lead to increased fruit abortion in *Turnera velutina*?		In prep.
2017	Publication	Peer-reviewed	The role of ants in papaya's (*Carica papya*) pollination		In prep.
2017	Publication	Peer-reviewed	Global levels of florivory		In prep.

References

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