Australia document draft report

**Introduction**

* *The wasps*
* *Physiology*
* *Community*
* *Climate*

Malvantheran figs and wasps…

Taxonomical classification of the diverse clade of fig wasps has been a dynamic field of study, with various revisions and reclassifications over the years (refs). Moreover, identification of the minute fig wasps to the species level using morphological characters alone is prone to mistakes, due to the existence of cryptic species which appear identical to the non-expert’s eye, as well as the lack of publicly available identification keys (ref). Despite the lack of clarity in the understanding of fig wasp taxonomy and true diversity, wasps associated with figs can be classified into three major functional groups: the pollinators, large non-pollinating galling wasps, and small parasitic wasps, also non-pollinating. Although most fig wasps belong to the family Agaonideae, some parasitic wasps outside the family can also be associated with the figs (refs). The fig wasp community associated with the figs can have many trophic levels, with parasitoids and hyperparasitoids (parasitoids of the parasitoids), and there is still some uncertainty to the functional role of some genera in the community and the ecological relationship between some species.

The pollinating wasps all belong to the subfamily Agaoninae:, phytophagous etc… Australian

Among the large non-pollinating fig wasp gallers associated with Malvanthera figs are the members of the subfamilies Epichrysomallinae and Sycophaginae (Agaonideae). Like the pollinators, these wasps are phytophagous, with developing larvae forming galls where the fig seed would otherwise develop. However, unlike the pollinators, oviposition happens from the outside of the fig. These non-pollinating wasps are often significantly larger than the pollinators and their emergence from the fig can alter development of the pollinator larvae, while foundresses might be competing with the pollinators for florets available for oviposition (ref). Diversity of the subfamily Epichrysomallinae in Australia spans across several taxa, with the genus *Meselatus,* in particular, having three species in Australia (*M. fasciatipennis, M. ficus and M. leai*), with *M.* *fasciatipennis* reported to be reared from *F. macrophylla* (Girault, 1922, 1929; Dodd, 1924; Bouček, 1988)*,* while the genus *Herodotia* has two species in Australia (*H. procopii* and *H. subatriventris*); (Girault, 1923, 1931; Bouček, 1988). The subfamily Sycophaginae includes the genus *Pseudidarnes*, with one species encountered in Australia (*P. miverva*), which is associated with *F. rubiginosa* (Girault, 1927; Bouček, 1988).

Parasitic wasps associated with the figs use other wasps as their host. They can oviposit either inside the pollinators, other galler wasps, or even other parasites (hyperparasitoids) (refs). Fig wasp parasites associated with Malvanthera figs include the subfamily Sycoryctinae (Agaonideae), and some members of the family Eurytomidae. The representatives of Sycoryctinae are generally agreed to be parasitoids of other wasps, most frequently laying their eggs inside fig gallers (refs). *Sycoscapter* and *Phylotrypesis* are believed to be parasitoids of the pollinating wasps, while *Watshamiella* is believed to be a hyperparasitoid of *Sycoscapter* (ref). The genus *Phylotrypesis* has five species in Australia (*P. angela, P. aterrima, P. immaculata, P. longiventris* and *P. silvensis*); (Saunders, 1883; Girault, 1915; Bouček, 1988) and the genus *Watshamiella* has one species in Australia (*W. aurea*), however more recent studies have revealed the existence of at least two morphospecies in *F. rubiginosa* alone (ref). The genus *Sycoscapter* has at least five species in Australia (*S. australis, S. huberi, S. miltoni, S. subaeneus* and *S. varicilia*). However, like *Watshiamiella*, more recent studies have revealed further diversity, and in the case of *Sycoscapter*, molecular data suggest that the group is not monophyletic, while taxon diversity can be higher than initially thought due to diversity in morphological traits (ovipositor length) within wasps attaching the same host fig (Segar *et al.*, 2012). The family Eurytomidae has one genus commonly associated with Malvanthera figs, the genus *Sycophila*. Like other members of the family, *Sycophila* is believed to be a parasitoid of galler Epichrysomallinae wasps (ref). unlike the Agaonideae parasitoids, which are usually small, with long external ovipositor, the Eurytomidae parasitoids are larger and typically have a coiled ovipositor (ref).



**Methods**

*Insect collection*

Fresh figs of the plant hosts *F. rubiginosa* and *F. macrophylla* were collected in and around Brisbane, South East Queensland, between November and December 2024. Figure X shows the sampling sites, located in and around Brisbane. The figs were collected at the ripening stage when we would expect emergence to happen (stage assessed using the colour and texture of the figs) and temporarily stored in plastic containers with customised ventilation using either a mesh net or an aluminium net. Wasps were allowed to emerge naturally after collection, and were kept in their original container until preparation for the experiments. Table X shows the exact time and location of the collection. In total, 22 figs from 10 trees had wasps emerging, usually during the night or early morning after collection. Many other figs collected, did not lead to wasp emergence, either due to false assessment of the ripening stage, of for other, ecological reasons. In preparation for the experiments, the containers with the insects from each host were placed in a cold room for 15-20 minutes after emergence, to reduce activity and allow handling of the wasps, using a small paintbrush. Insects were then separated individually into empty 1.5ml Eppendorf tubes. Insects were kept in room temperature (22°C) for at least 30 minutes before any of the thermal tolerance experiments commenced.

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| A map of australia with a purple dot  Description automatically generated | A map of a city  Description automatically generated |

Figure X. final experiment only, all fig in supplementary.

*Insect identification*

The wasps were initially identified morphologically into three functional groups; these were the pollinators (with the typical morphology of *Pleistodontes* wasps), small NPFW (wasps like *Sycoscapter, Philotrypesis, or Watshamiella*) and large NPFW (typically galling wasps from the family Epichrysomallidaea). This rough classification was enough when separating wasps into treatment groups, however further morphological investigation was performed *a posteriori*, using digital photographs of the individual wasps to assign. Given the lack of clarity in species identification within Agaonidae, for the purpose of this study classification of wasps using the photographs was restricted to the genus level in most cases, using the generally accepted genus names. Presentation of these genera in the current study follows the classification in Bouček (1988), however it is worth noting that there have been more recent suggestions for the reclassification of the genera within new tribes/subfamilies (refs).

*Thermal tolerance experiments*

In all the experiments presented in this study, the temperature treatments were applied by placing the Eppendorf tubes with the wasps into dry heat blocks set at the desired treatment temperature. Three heaters were available in total (Grant instruments’ BTD Dry Block Heater for Microtubes, Benchmark’s Traditional Digital Dry Bath and SciQuip’s Digital Dry Block Heater). The heaters were allowed to come to treatment temperature prior placing the wasps into the blocks. Assessment of wasp activity for all experiments in the study was done by bringing individual tubes out of the block and tapping onto the bench. If the wasp started walking onto the tube walls, the status was recorded as active, while if the wasp remained immobile or had lost grip from the tube walls completely, the status was recorded as inactive, and the wasp was deemed dead.

To explore the optimal range of the experimental parameters for thermal tolerance, a few small-scale pilot experiments were performed first ~~A visual summary of the pilot studies setup is shown in figure X~~. (Supplementary data).

After assessment of the pilot study results, the main experimental setup was design as such: Wasps emerging from the same fig of either host species were haphazardly split into 3 treatment groups, with wasps from different functional groups separated proportionally into each group, and with each group having a balanced number of individuals in each run. Each group was haphazardly assigned to one of 3 treatments, each set at a constant temperature of 39°C, 41°C and 43°C, and all running simultaneously at every experimental round. Activity status was checked every 30 minutes. The number of wasps included in each experimental round depended on wasp emergence; all wasps emerging from a fig were used and the experiment was done in multiple rounds over different days, using different figs. The age of wasps exposed to treatments varied across the experimental rounds (wasps used were a few hours to a few days old).

Upon completion of the thermal tolerance experiment, photographs of the wasps were taken using a Dino-Lite digital microscope and the DinoXcope application (AnMo Electronics Corporation) after a digital measuring scale had been added to the frame. The wasps were kept in 70% ethanol and stored in the fridge (temp) until DNA extraction. To explore variation in response at the mid-temperature treatment, the head length measurements of all pollinating wasps exposed at 41°C were taken as a proxy of body size using the digital photographs. This was done by measuring the longest vector of the head at Fiji (Schindelin *et al.*, 2012), which due to the wasp morphology, was easier to standardise than any other body-size measurement in the photos.

*Barcoding*

*Statistical analysis*

Initially, a survival analysis was performed, using the combined information from the temperature treatment and functional wasp groups as the predictor. Analysis was done by combining information of the two hosts when applicable (by pooling all pollinators and small parasitoids together). Time of death was considered to be the time at the nearest 30-minute interval up since the wasp was last found active (for example, for a wasp which was alive at 30 minutes but dead at 60 minutes, time of death for the survival analysis was recorded as 60 minutes). Analysis was done by running a Cox regression model from the r package ‘survival’ to obtain the hazard ratios between our comparisons (Therneau, 2023). Following survival analysis, an ordinal regression was performed, this time using the temperature treatment, the functional group and the fruit of origin as independent predictors of death time (response variable). Death time was coded as an interval of six levels, representing death time between the 30-minute recordings, starting from the lower level representing deaths before 30 minutes, and up to the highest level representing deaths above 150 minutes, where the recordings stopped. Ordinal regression was performed using the r package ‘ordinal’ (Christensen, 2023). This was followed by a post-hoc test, using the r package ‘emmeans’ (Lenth, 2024), while the package ‘RVAideMemoire’ (Herve, 2023) was used to evaluate the test statistics. To explore the interaction effects of temperature and functional group in greater detail, the focus was then shifted at the status of the wasps at the end of the experiment. A logistic regression was performed, using the status of the wasp at the end of the experiment as the response variable, and using fruit of origin, temperature treatment, and functional group within each host as the predictors, including the interaction of the later two. This was done after model selection using Akaike Information Criterion (Bozdogan, 1987). The logistic model was done using the r package ‘brglm2’ (Kosmidis, 2023) to reduce estimation bias, and the McFadden's pseudo R-squared value was estimated (McFadden, 1974). A post-hoc test was performed using the r package ‘lsmeans’ (Lenth, 2016). Finally, to explore the variation in pollinator wasp survival observed at 41°C, logistic regression models were produced, one for each pollinator species, using survival at the end of the experiment as the response variable, and wasp head length as the predictor. All statistical analyses were performed in RStudio v.2024.4.0.73 (Posit team, 2024).

**Results**

*Community composition*

The majority of wasps emerging from the figs were the *Pleistodontes* pollinators (40.0% *P. froggattii* and 35.2% *P. imperialis*). However, these were not equally distributed in the figs; one third of figs produced non-pollinating wasps only, with *Sycophila,* *Meselatus,* and *Herodotia* having the highest occurrence rate among those figs (Fig. X). For the whole experiment, the large non-pollinating fig gallers were only found in *F. rubiginosa* figs. These were mostly *Meselatus* wasps, while *Herodotia* and *Pseudidarnes* were rarer (Figs. X and X). Small non-pollionating parasitic wasps were found in both *F. macrophylla* and *F. rubiginosa* figs; among these, *Sycoscapter* was the most abundant, making up 8.3% and 6.0% of all wasps found in each host respectively. While the *Sycoscapter* wasps in *F. macrophylla* are all most likely *S. australis*, the *Sycoscapter* wasps of *F. rubiginosa* likely belong to at least two distinct morphospecies, one with short and one with long ovipositor. While *Watsamiella* was found in both hosts, albeit in small numbers, *Phylotrypesis* was only found in *F. rubiginosa*. Large parasitoids, specifically *Sycophila,* was only found in *F. macrophylla*.



Figure X. Summary of wasp distribution as found in host figs. The first row (fruits UQMA1 to VPMA3) represents figs of *F. macrophylla*, while rows two and three (fruits UQRU2 to UQRU13) represent figs of *F. rubiginosa*. Numbers represent individuals emerged from each fig. Taxa were identified using photographs.

*Thermal tolerance*

Overall, survival of wasps was significantly reduced above 41°C, while further reduction in survival was also noticeable above 43°C. Since all large gallers exposed to 39°C were alive by the end of the experiment, they were excluded from the survival analysis. The proportional hazard ratio (HR) regression showed that differences in survival do exist among the groups (Likelihood ratio test = 350.8, df = 10, n= 512, p < 0.001). Around half of all wasps were found dead by the end of the experiment (number of events= 260). The small parasitoids of the two hosts combined had the smallest HR for both the 39°C and 43°C exposure treatment, while large parasitoids and all *Pleistodontes* pollinators had the highest HR for both the 41°C and 43°C treatments (Fig. X). Although there is significant overlap of the confidence intervals in most comparisons, all functional groups have significantly higher hazard ratios at 43°C compared with the baseline of the experiment at 39°C. Within each temperature treatment, the only significant difference was observed between pollinators and small parasitoids at 43°C, with pollinators having a higher HR.



Figure X. Cox proportional hazards survival analysis. Pollinators exposed to 39°C were used as the reference group. Larger hazard ratio (x-axis) implies a higher death hazard. Sample sizes, hazard ratios and 95% confidence intervals are shown next to each group. P-values are shown at the far right of each group, where significance is drawn from comparison to the reference group.

Results from the ordinal regression where the main effects of functional group, treatment and fruit were explored as predictors of death time, mirrored survival analysis (Fig. X). All predictors were found to have a significant effect on wasp death time (logLik = -419.59, df = 14, n = 519, p < 0.001). Since the wasps of each functional group of each host were split for the ordinal regression, post hoc results could pinpoint differences in death time within each host. All temperature treatment comparisons were found to be significant, revealing faster deaths at higher temperatures, while only some of the functional group and fruit combinations were significant (supplementary).



Figure X. Proportion of active (alive) individuals at each time interval monitored for wasps from the two hosts and for the three temperature treatments. Functional groups are represented with different colours, while sample size of wasps at each category are shown for Time = 0.5h (same individuals monitored across time).

When focusing on wasp survival the end of the experiment only, the interaction effects of treatment and functional group started emerging. The binomial model could explain almost half of the variation in our data (McFadden's pseudo R-squared = 0.52), while the post hoc tests pinpointed differences within hosts (supplementary). Notably, the survival analysis results were confirmed for the two hosts independently, as both pollinator taxa had significantly reduced survival for each treatment comparison. Additionally, for *F. rubiginosa* wasps, the small parasitoids had elevated survival rate at 43°C compared to the pollinators, but not for the lower temperature treatments. Large galler survival was not different to the survival of pollinators and small parasitoids. The wasps from *F. macrophylla* showed a more uniform pattern of survival across the functional groups. Due to large parasitoids only been found in two fruits exclusively, direct comparison with other functional groups was not possible. However, their own survival was not found to be affected significantly by temperature either, which is possibly an effect of their low sample size (7 individuals in each treatment).



Figure X. …..

Comparison of survival at the end of the experiment across different fruits was only possible where fruits had a similar assembly of wasps (representing the same functional groups). Although fruit of origin had a significant effect in wasp survival (supplementary), this was only true for comparisons within *F. macrophylla*.

Since at the 41°C treatment the chance of survival was around 48.92% for all wasps, further exploration of morphological data as potential predictors of survival were used. For this hypothesis, we only focused on the pollinators, *P. froggattii* and *P. imperialis*, which had 47.89% and 57.58% mortality rates respectively by the end of the 41°C treatment. The logistic regression using wasp head length as predictor of wasp survival did not show any significant effect for neither taxa (*P. froggattii:* chi sq. = 0.17215, df = 64, p = 0.6782; *P. imperialis:* chi sq. = 2.5655, df = 67, p = 0.1092), despite the small trend of increased survival of larger *P. imperialis* (Fig. X).



Figure X.

**Discussion**

Notes:

Comparison of thermal tolerance of pollinators: in my results: smaller individuals have higher surface area-to-volume ratio, maybe better for heat loss.

Things to keep in mind: behavioural adaptations vs canopy flight; darker colouration might be a disadvantage in the wild;

Because of lack of certainty on the species identification, and therefore overall diversity in the experimental dataset.

**Conclusion**

**References**

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**Supplementary data**

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| --- | --- | --- | --- |
| 1. Fig wasp community of sampled *F. macrophylla* figs: | | | |
| 1) Pollinators: | 2) Large parasitoids: | 3) Small parasitoids: | |
| Agaoninae | Eurytomidae | Sycoryctinae | |
| A close up of a bug  Description automatically generated  *Pleistodontes froggattii* | *A close up of a black insect  Description automatically generated*  *Sycophila* sp | *A close up of a bug  Description automatically generated*  *Sycoscapter australis* | A bug with wings and a long thin tail  Description automatically generated  *Watshamiella* sp |
|  | | | |
| B) Fig wasp community of sampled *F. rubiginosa* figs: | | | |
| 1) Pollinators: | 2) Large gallers: | | |
| Agaoninae | Epichrysomallinae | | Sycophaginae |
| A bug with wings and a bug on it  Description automatically generated  *P. imperialis* | A close up of a bug  Description automatically generated  *Meselatus* sp | A close up of a bug  Description automatically generated  *Herodotia* sp | *A close up of a bug  Description automatically generated*  *Pseudidarnes minerva* |
| 3) Small parasitoids: | | | |
| Sycoryctina | | | |
| A close up of a bug  Description automatically generated  *Phylotrypesis* sp | *A close up of a bug  Description automatically generated*  *Watshamiella* sp | *A close up of a bug  Description automatically generatedSycoscapter* sp long | *A close up of a bug  Description automatically generatedSycoscapter* sp short |

*Pilot experiments*

In the first pilot study, wasps emerging from a single fig from a F. rubiginosa host were exposed to either of two temperature treatments, 40°C or 45°C , and the activity status of the wasps was assessed every 5 minutes for a total of 25 minutes. For each treatment, 11 pollinators and 1 small NPFW were used. The aim of pilot study 1 was to provide a first rough estimate of the temperature range and duration of exposure that would be meaningful to test for wasps from the host F. rubiginosa.

In the second pilot study, pollinating wasps from the same single fig from the same F. rubiginosa host were exposed to changing temperature conditions. Half of the wasps (10 individuals) were exposed to temperatures between 40°C and 43°C, with the temperature increasing by 1°C every 20 minutes, and activity assessment done every 10 minutes. The remaining half of the wasps were exposed to temperatures between 40°C and 45°C, with the temperature increasing by 1°C every 10 minutes, and activity assessment done every 10 minutes. Both treatments stopped when all wasps were inactive. The aim of pilot study 2 was to estimate if a rapid rate of temperature increase would affect wasp activity more than the temperature of exposure itself.

In the third pilot study, pollinating wasps from the same single fig from the F. rubiginosa host were exposed to either of two temperature treatments. The 10 wasps included in the first treatment were exposed to changing conditions, kept at 41°C for 20 minutes, they will then brought to room temperature for another 20 minutes, and were finally brought back into the heater at 41°C for 20 minutes. The second treatment (10 wasps) was constant exposure to 41 °C for 1h. The activity status of the wasps in both treatments was assessed every 20 minutes. The aim of pilot study 3 was to provide a comparison of constant exposure and interrupted exposure to suboptimal temperatures.

In the fourth pilot study, wasps from a single fig from a F. macrophylla host were exposed to either of three temperatures. Treatments were 39°C, 41°C or 43°C, and exposure for 4h and 20 minutes. Each treatment included 10 pollinators and 1 small NPFW. The aim of pilot study 4 was to provide a rough estimate of the temperature range and duration of exposure that would be meaningful to test for the wasps of the second fig host F. macrophylla.

In the fifth pilot study, wasps from the same single fig from a F. macrophylla host were exposed to changing temperature conditions. Wasps (20 pollinators and 2 small NPFW) were exposed to temperatures between 35°C and 44°C, with the temperature increasing by 1°C every 5 minutes and activity status being checked every 10 minutes. Half the wasps remained in the heater until the end of the experiment, while the remaining half were brought to room temperature every 5 minutes, for a duration of 5 minutes, while they remained in the heater in between. Both treatments stopped when most wasps from each treatment were inactive. The aim of pilot study 2 was to estimate if return to room temperature would enable wasp recovery from the thermal stress, despite increasing temperatures of exposure.