The effect of crab spider presence on pollinator behaviour in the field.

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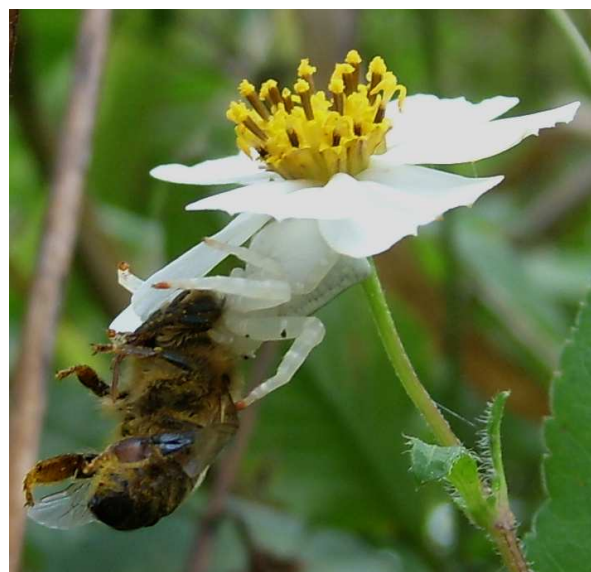Felipe Gawryszewski

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Summary

Some Australian crab spiders reflect UV light and create a strong colour contrast against their UV-absorbing flower background. This contrast has been shown to be attractive to honeybees, but not to native Australian pollinators in laboratory - and semi-field situations. In this research, the effect on UV-reflecting and UV-absorbing crab spiders on pollinator behaviour is tested in the field. Honeybees were found to be attracted to flower patches containing spider models creating a colour contrast. Within the patch, they however showed no preference for vacant flowers or flowers occupied by crab spiders.

Introduction

Crab spiders (Araneae: Thomisidae) are ambush predators that use flowers as hunting sites (Heiling and Herberstein 2004a, Morse 2007). They feed on a wide range of pollinating insects, such as honeybees, bumblebees, butterflies and syrphid flies. They do not build a web, but position themselves on a flower or between two inflorescences with only their characteristically long forelimbs sticking out (Fig. 1a). They wait for pollinators to approach, which they then grab with these forelimbs (Morse 2007) (Fig. 1b). Subsequently, the spider injects venom and enzymes into the prey in order to break down and imbibe its tissue (Morse 2007, Pollard 1989).

Figure 1a: Thomisus spectabilis *on* Lantana camara. *Picture by Felipe Gawryszewski.*

Figure 1b: Thomisus spectabilis *on* Tanacetum sp.. *Picture by Felipe Gawryszewski.*

In order to find a suitable hunting site, crab spiders make use of similar cues as pollinators use when searching for a rewarding flower. Heiling and Herberstein (2004b) found that, similar to honeybees, crab spiders prefer flowers which offer a greater pollen resource over less rewarding flowers and make use of visual, tactile and especially olfactory cues to differentiate between flowers (Heiling *et al*. 2004, Wignall *et al*. 2006). By doing this, crab spiders exploit the signals used by flowers to attract pollinators in order to identify a flower with a high chance of prey encounter.

European crab spiders are generally well camouflaged on their flower background to prevent being detected by pollinator prey (Théry and Casas 2002). However, some Australian crab spiders use a different approach. *Thomisus spectabilis* and *Diaea evanida* occur in two colour morphs, yellow and white, and can change their body colour between these two morphs (Heiling *et al*. 2005a). Yellow spiders prefer to hunt from yellow flowers, while white spiders do not seem to have a preference and are found on both white and yellow flowers (Heiling *et al*. 2005a).

Through human vision, white spiders seem perfectly camouflaged on white flowers. However, white spiders reflect UV-light, while their flower backgrounds do not. Thereby, the spiders create a strong colour contrast, which is visible through insect vision (Heiling *et al*. 2003, Herberstein *et al*. 2008) (Fig. 2). Surprisingly, this contrast is attractive to foraging European honeybees. Heiling and Herberstein (2003) showed that honeybees preferred a white flower occupied by a white spider over a vacant white flower. An explanation for this behaviour is that insects are naturally biased towards UV light (Briscoe and Chittka 2001, Herberstein *et al*. 2008). Furthermore, bees are attracted to strongly contrasting marks on flowers, as these can serve as nectar guides (Lunau *et al*. 1996, Lunau 2000). Thus, crab spiders that create a UV colour contrast might be mistaken as nectar guide on a flower and thereby attract pollinators.

When white spiders were treated with a UV-absorbing chemical, causing them to not reflect any UV light, honeybees were deterred by the presence of the spiders and now preferred vacant daisies (Heiling *et al*. 2005b). Similar to European honeybees, Australian native bees (*Austroplebia australis*) are attracted by the contrasting colour signal created by crab spiders that reflect UV light. However, these native bees do not actually land on flowers occupied by crab spiders but prefer to land on vacant flowers (Heiling and Herberstein 2004a). As native bees have evolved together with Australian crab spiders, it is likely they developed anti-predatory behaviour, unlike European honeybees, which were only introduced

in Australia about 200 years ago (Heiling *et al*. 2006). The means by which native bees can perceive crab spiders on flowers are however not known.

Yellow *Thomisus spectabilis* do not reflect UV light and honeybees as well as native bees do not have a preference for either spider-occupied or vacant flowers. Heiling *et al*. (2005a) suggested white crab spiders have an advantage in hunting on the introduced honeybee, while yellow crab spiders have an advantage in hunting on native prey. This hypothesis has however not been tested yet.

Although interesting results have been observed under laboratory and semi-natural conditions, little or no data are available yet on the interaction between UV-reflecting crab spiders and pollinators in the field. Literature on field studies is available for the crab spider species *Misumena vatia*. Dukas and Morse (2003, 2005) reported in several field studies that fewer honeybees visited patches where crab spiders were present than patches without crab spiders. *Misumena vatia* is however UV-absorbing and does not create an attractive colour contrast on its flower background.

Figure 2: A white Thomisus spectabilis *crab spider on a white daisy, as visible with human colour vision (left) and as visible with honeybee colour vision (right) Source: Heiling and Herberstein 2004b*

The aim of this study is to investigate the interaction between visiting pollinators in the field and both white UV-reflecting and yellow UV-absorbing crab spiders on yellow and white flower backgrounds. It is expected that honeybees, which are non-native in Australia, will be attracted to flowers harbouring white spiders, while native pollinators will prefer to land on either vacant flowers or yellow flowers harbouring yellow crab spiders. All pollinators will be deterred by yellow spiders on white flowers. It is therefore predicted that white crab spiders will be more successful in attracting honeybees, while yellow crab spiders on yellow flowers will be more successful in attracting native pollinators.

Materials & Methods

Plants and insects

Yellow Cosmos (*Cosmos sulphurous* Cav.) flowers were freshly cut on each experiment day in fields in Sydney and placed in bottles with water and flower preservative ("Bell Fleur", Hortipack BV, Roosendaal the Netherlands). Only yellow flowers were used in this experiment, as insufficient suitable white flowers were present in the area for the experiment.

White *Thomisus spectabilis* crab spiders used for the experiment were collected in Airlie Beach, Queensland in 2008 and *Diaea evanida* crab spiders were collected in Sydney in 2008 and 2009. Both species were kept in plastic cups (diameter 8 cm on the bottom, 5 cm on the top and 10 cm in height) in the laboratory of Macquarie University, Sydney, on a 12:12 hours light cycle and temperatures ranging from 20 to 25°C. They were fed live houseflies and fruit flies (*Musca domestica* and *Drosophila melanogaster* resp.) and were watered daily.

Diaea evanida was found unsuitable for the experiment as the majority of the spiders quickly escaped from the patch. Plasticin spider models (Fig. 3) were used instead to eliminate the effect of spider behaviour and to standardise size and shape.

Figure 3: Yellow plasticin spider model on Cosmos flower with visiting honeybee. Picture by author.

In order to generate realistic models, the colour of 36 live *D. evanida* spiders was analysed with an OOIBase32 spectrophotometer (Ocean Optics). Then, plasticin colours were mixed to match the colour of white UV-reflecting and yellow UV-absorbing *D. evanida* spiders. The yellow spider models were painted with sunscreen to block UV reflection, as all plasticin was found to reflect UV-light. The spider models were subsequently sprayed with Plasticote 70 clear protective lacquer (CRC, Australia) to block the odour of the plasticin and the

sunscreen. The spider models all had an equal weight that corresponded with the average weight of the real *D. evanida* spiders measured. Live *Thomisus spectabilis* were found to stay within the patch throughout the experiments and it was therefore not necessary to obtain plasticin models for this species.

Experimental setup

The bottles containing fresh Cosmos flowers were placed in a tray. Each tray fitted with 20 flowers at a distance of 8 cm between individual flowers represented one patch. Ten spiders or spider models were randomly placed in each patch. Four trays (one with yellow spider models, one with white spider models, one with real *T. spectabilis* spiders and one with vacant flowers only) were placed in field sides containing naturally occurring Cosmos flowers at a distance of 60 cm between the trays. Digital cameras were placed in such a way that each camera recorded one patch from approximately 1m above. Cameras monitored the activity in the patches between 9 am and 6 pm. Each day, the experiment was set up in a different field side and the placement of the patches was randomised.

From the video material, the number of visiting pollinators within the patch was counted as well as number of visits to individual flowers, time spent within the patch, time spent on the individual flower and spider response.

Statistical analysis

All results had a non-normal distribution. Since over 90% of the visiting pollinators were honeybees (*Apis melifera*), statistical analyses was only done for this species.

Between-patch-analyses: The number of honeybee visits per patch was analysed with a chi-square test. The average amount of time spent within the patch and on the flower was analysed with a Kruskal-Wallis test.

Within-patch-analyses: The patch containing *Thomisus spectabilis* was excluded from these analyses as it was not possible to tell at all times whether a flower was vacant or occupied, since the spiders moved around a lot and frequently hid underneath the flower. The number of visits on vacant and occupied flowers was compared for the patches with model spiders using a Wilcoxon matched-pair signed-rank test. The average amount of time spent on the vacant and occupied flowers was analysed with a Mann-Whitney U test.

Results

The effect of white UV-reflecting and yellow UV-absorbing crab spiders on a UV-absorbing flower background on pollinator behaviour in the field was investigated. In total, 105 pollinator visits were recorded in the experimental sides during three days, consisting of 91 honeybee visits, four butterfly visits and 10 syrphid fly visits. No native bees were observed within the patches. The butterflies and syrphid flies were excluded from the analysis as their numbers were insufficient to make correct conclusions. On four experimental days, insufficient pollinators were present, mainly due to bad weather conditions.

Spectral reflectance curves

The reflectance of the plant and insect material used in the experiment was quantified with a spectrophotometer (Ocean Optics). White *Diaea evanida* as well as white spider models showed between 40 and 50% reflectance of light between 500 and 700 nm ((Fig. 4). The spider models reflected however more strongly in the UV region (300 – 400 nm) than live *D. evanida*.
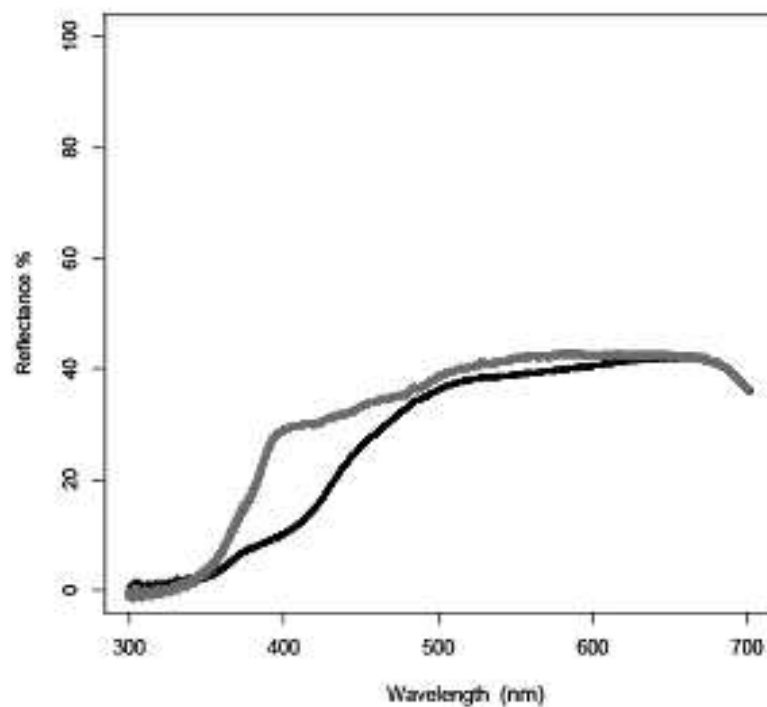

Figure 4a: Percentage spectral reflectance of live white Diaea evanida
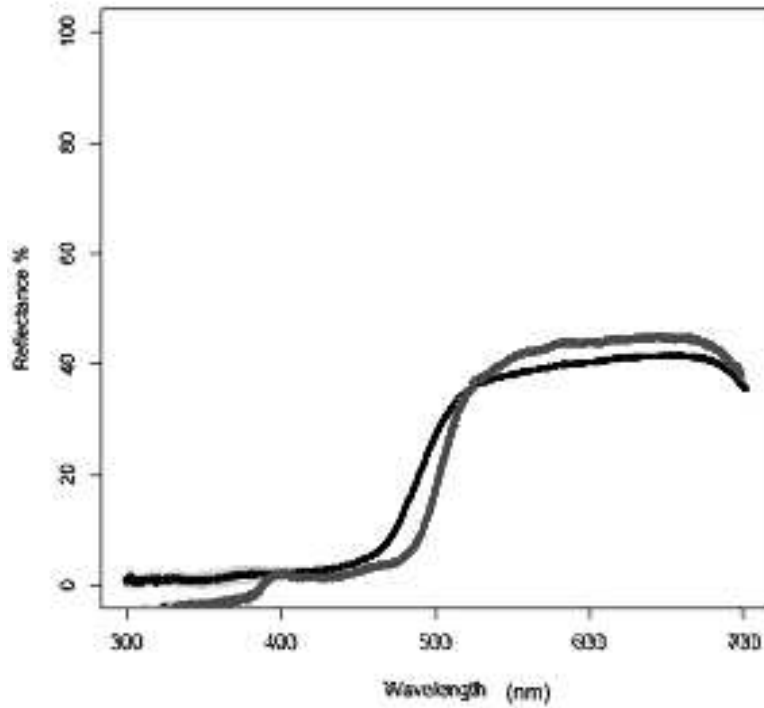*(black curve) (N = 9) and white spider models (grey curve) (N = 8).*

Figure 5: Percentage spectral reflectance of live yellow Diaea evanida *(black curve) (N = 10) and yellow spider models (grey curve) (N = 8).*

Both yellow *Diaea evanida* and yellow spider models showed no reflection in the UV region (Fig. 5). The spider models showed less reflectance than live *D. evanida* at 500 nm, and slightly more from 530 – 700 nm.
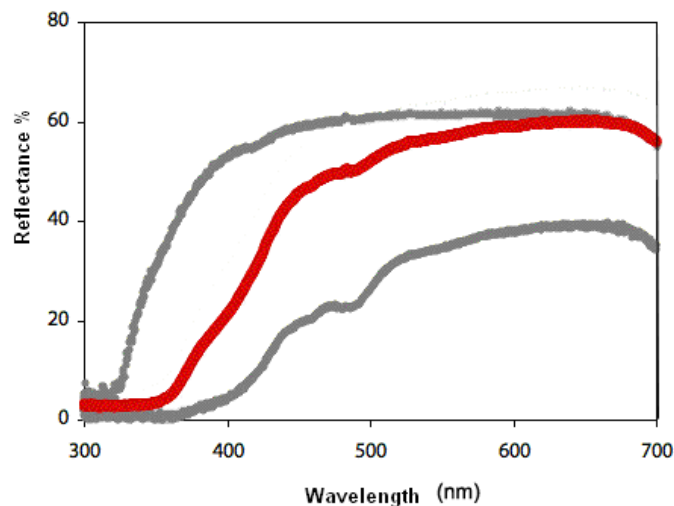

Figure 6: Percentage spectral reflectance of live Thomisus spectabilis *showing the maximum and minimum range and the average reflectance. N = 76*

Different *Thomisus spectabilis* individuals showed a great spread in light reflectance (Fig. 6), ranging from less than 10% to over 50% in the UV region and about 40 % to 60% in from 450 – 700 nm.

Yellow Cosmos flowers showed no reflectance in the UV region and about 60% reflectance from 550 – 700 nm (Fig. 7)
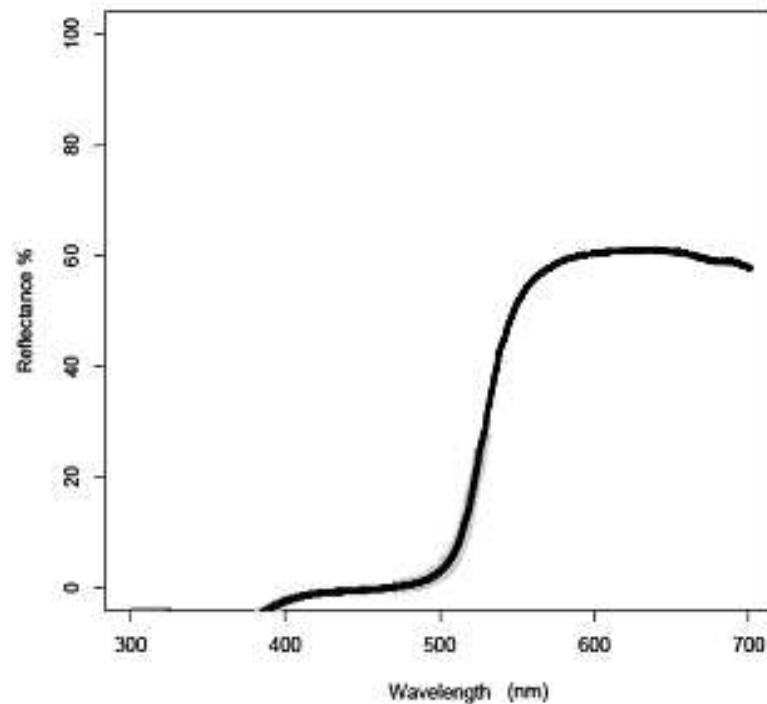

Figure 7: Percentage spectral reflectance of Cosmos sulphurous *flowers.*
N = 116

Between-patch analyses

The number of honeybee visits differed significantly between the four different patches (vacant and containing either *Thomisus*, white or yellow spider models) (Chi-Square Test: χ^2 = 19.636, p < 0.001, N = 88) (Fig. 8). In order to determine which patches differed from each other, individual Chi-Square tests were performed and a Bonferroni correction was applied, lowering the alpha value to 0.05/4 = 0.0125 for four comparisons.

No significant difference was observed in number of honeybee visits between the vacant patch and the patch containing *T. spectabilis* (Chi-Square Test: χ^2 < 0.001, p = 1.000, N = 24). Significantly more honeybee visits were observed in the patch containing white spider models than in the vacant patch (Chi-Square Test: χ^2 = 12.000, p = 0.001, N = 48). When comparing the vacant patch and the patch containing yellow spider models, significantly more honeybee visits were observed in the patch containing yellow spider models than in the vacant patch

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(Chi-Square Test: $\chi^2 = 6.400$, p = 0.011, N = 40), though the difference in number of visits was less pronounced than when comparing the vacant patch and the patch containing white spider models (Fig. 8). No significant difference was observed in number of honeybee visits between the patch containing white spider models and the patch containing yellow spider models (Chi-Square Test: $\chi^2 = 1.000$, p = 0.317, N = 64).
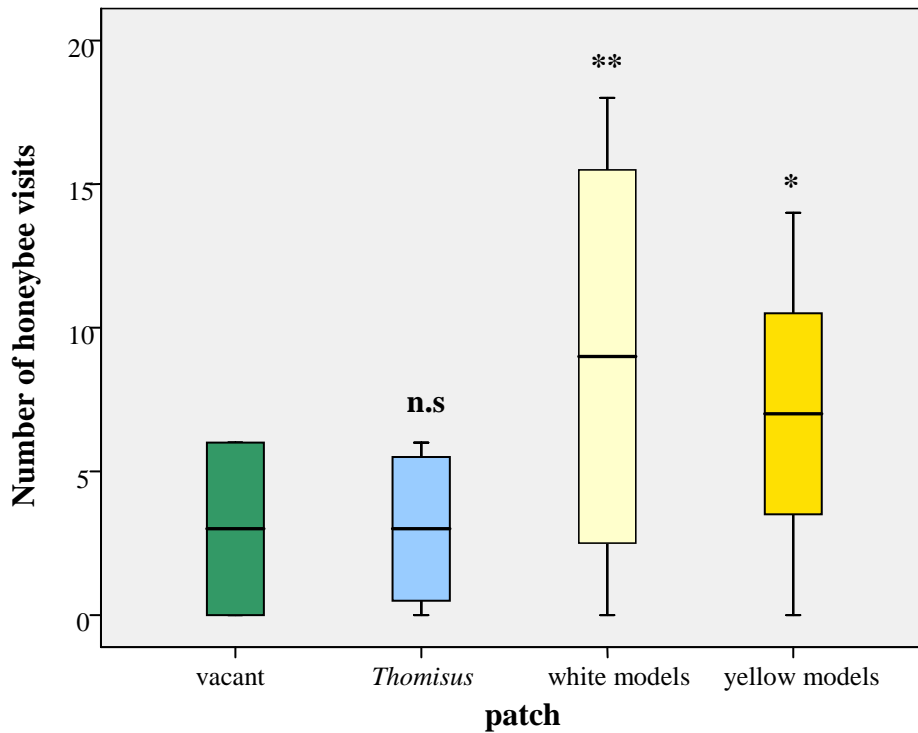

Figure 8: Box plots of honeybee visits per patch (vacant, containing T. spectabilis *or either white or yellow spider models). n.s. = not significant, * = p < 0.0125, ** = p < 0.01.*

No significant difference was found in the amount of time a honeybee spent in a given patch between the four different patches (vacant and containing either *Thomisus*, white or yellow spider models) (Kruskal Wallis test: $\chi^2 = 4.796$, p = 0.187, N = 86) (Fig. 9).

No significant difference was found in the median time a honeybee spent on a flower between the four different patches (Kruskal-Wallis test: $\chi^2 = 4.857$, p = 0.183, N = 86) (Fig. 10).
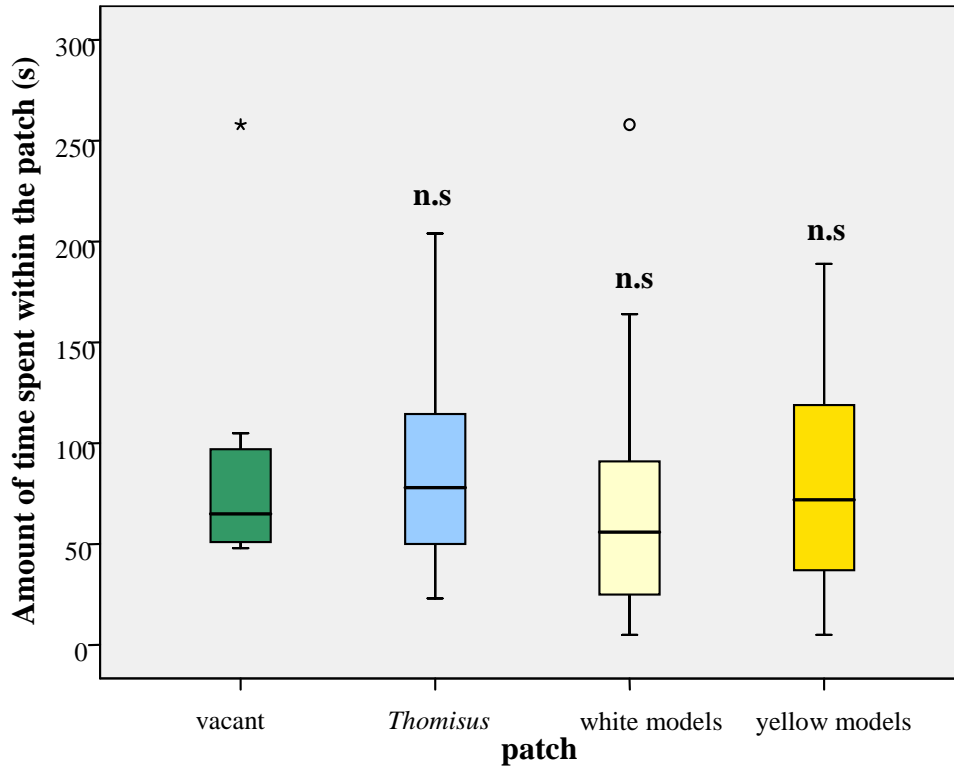
Figure 9: Box plots of the amount of time spent within the patch (vacant, containing T. spectabilis *or either white or yellow spider models) by honeybees. n.s. = not significant.*
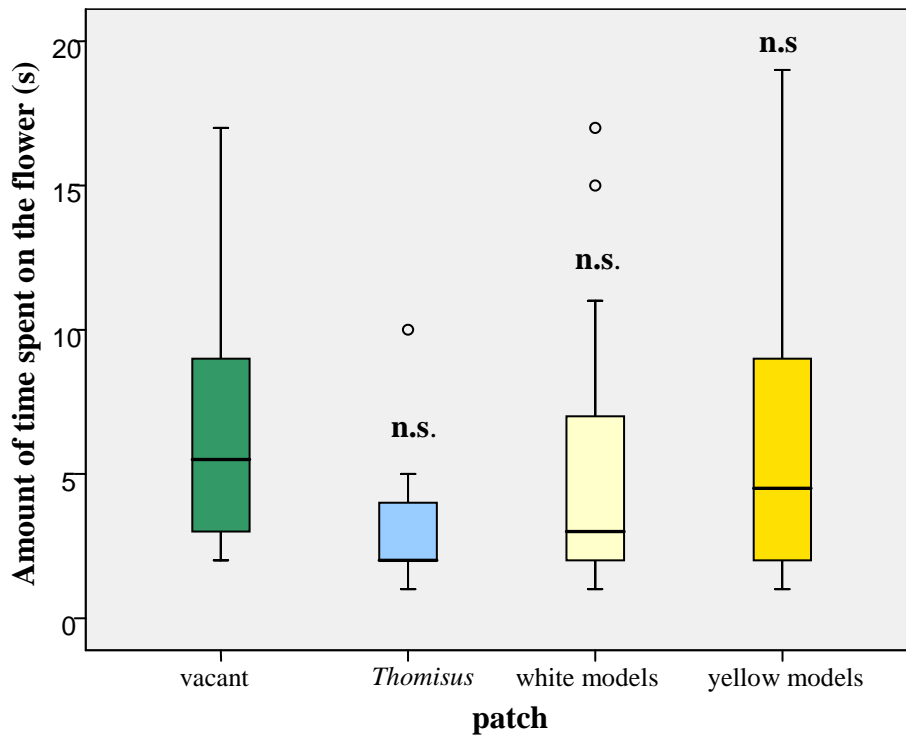

Figure 10: Box plots of the amount of time spent by honeybees on individual flowers within each patch (vacant, containing T. spectabilis *or either white or yellow spider models). n.s. = not significant.*

Within-patch analyses

Within the patch containing white spider models, no significant difference was found in number of honeybee visits between vacant and occupied flowers (Wilcoxon Matched-Pair Signed Ranks test, Z = -0.041 ; p = 0.967; N = 36) (Fig. 11). Within the patch containing yellow spider models, honeybees visited vacant flowers significantly more often than occupied flowers (Wilcoxon Matched-Pair Signed Ranks test, Z = -2.225; p = 0.026; N = 28).
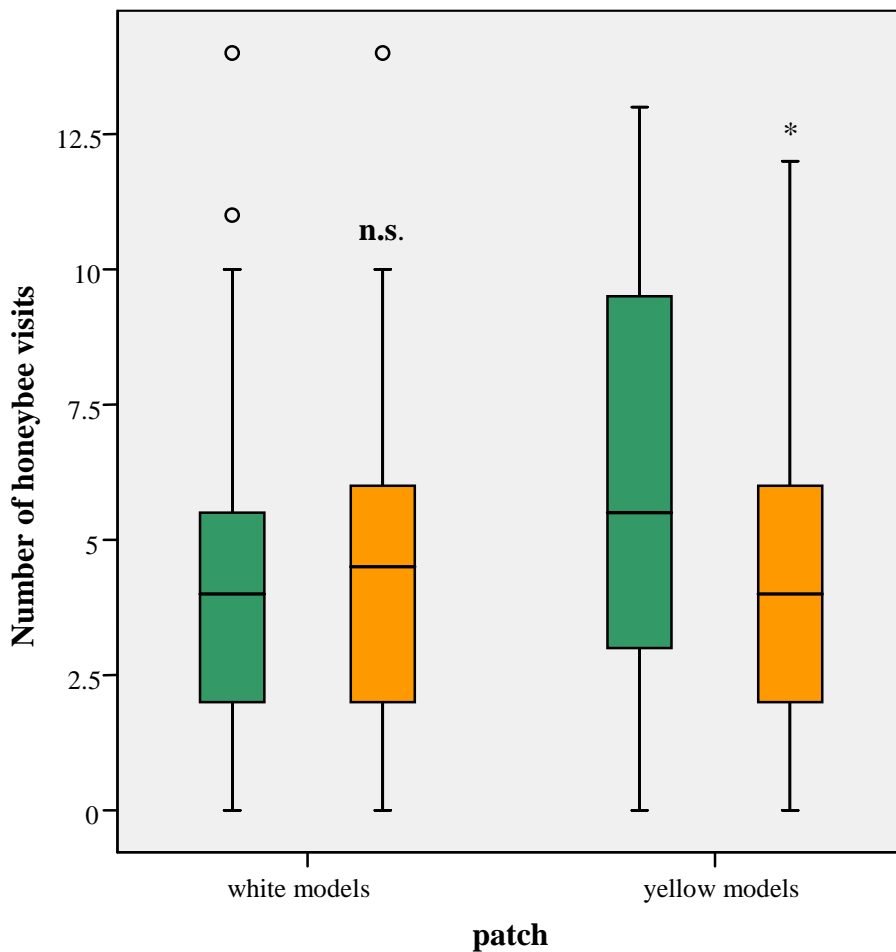
*Figure 11: Box plots of honeybee visits on either vacant flowers (green boxes) or flowers occupied by a white or yellow spider model (orange boxes). "White models" refers to the patch containing white spider models, "yellow models" refers to the patch containing yellow spider models. n.s. = not significant, * = p < 0.05.*

Within the patch containing white spider models, no significant difference was found in median amount of time spent by honeybees on vacant flowers or flowers occupied by a spider model (Mann-Whitney U: Z = -0.732, p = 0.464, N = 67) (Fig. 12). Also, no significant difference was found when comparing the amount time spent on vacant flowers and flowers

occupied by a spider model within the patch containing yellow spider models (Mann-Whitney U: Z = -0.307, p = 0.759, N = 49).
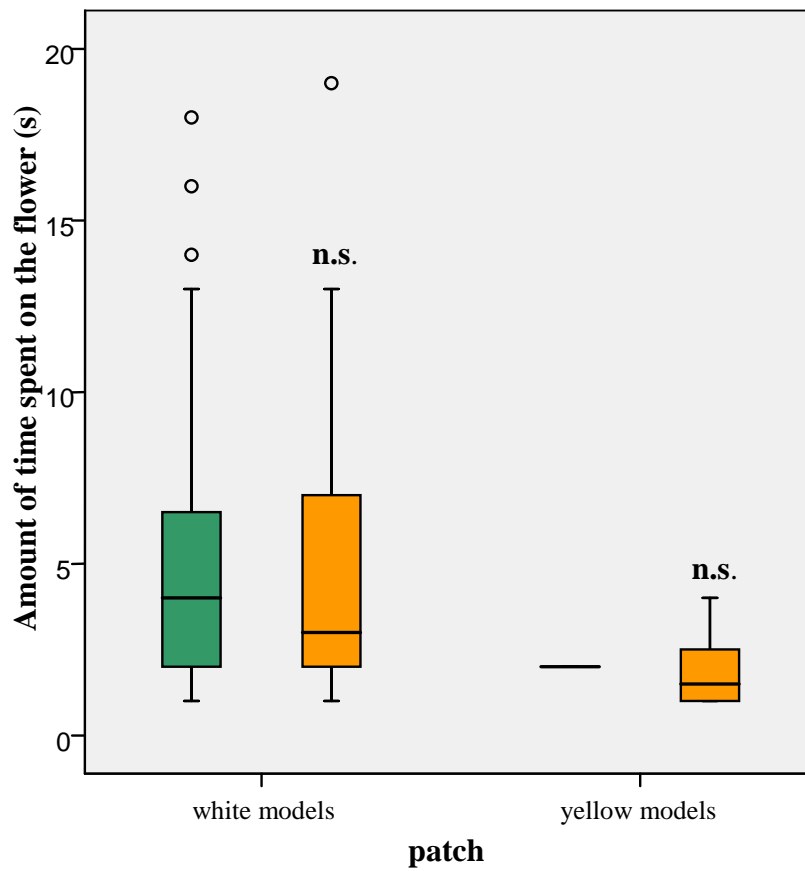

Figure 12: Box plots of the amount of time spent by honeybees on either vacant flowers (green boxes) or flowers occupied by a white or yellow spider model (orange boxes). "White models" refers to the patch containing white spider models, "yellow models" refers to the patch containing yellow spider models. n.s. = not significant.

Discussion

Between-patch analyses

Honeybees discriminated between the four different Cosmos flower patches (vacant and containing either white *T. spectabilis*, white or yellow spider models) in terms of number of visits (Fig. 12). The number of honeybee visits was similar for the vacant patch and the patch containing *T. spectabilis*, but significantly more honeybee visits occurred in the patches with spider models. The effect observed for the patch containing white spider models corresponds with the hypothesis that honeybees will be attracted to flowers harbouring white spiders, as the white spiders are UV-reflective and create a colour contrast against the UV-absorbing flower background, which is attractive to honeybees (Heiling and Herberstein 2003). However, the same effect was expected for the patch containing life white *T. spectabilis*, as Heiling and Herberstein observed that honeybees preferred a flower with a white crab spider over a vacant flower. A possible explanation for the different result in this study is that very frequently, the spiders chose to remain hidden underneath the flower. This caused the patch to look similar as the patch consisting of vacant flowers. In the experiment performed by Heiling and Herberstein, the spider was anaesthetised and thus remained visible on top of the flower.

No significant difference in number of visits was found between the patch containing white and the patch containing yellow spider models. This is surprising, as the yellow spider models were UV-absorbing and were thus not supposed to be attractive to honeybees. However, despite the efforts to make the reflectance spectrum of the plasticin spider models as much as possible similar to that of real *Diaea evanida* spiders, the spider models showed a colour brightness corresponding to that of the brightest *D. evanida* spiders measured, instead of corresponding with the average brightness of all spiders (results not shown). Honeybees were possible attracted to the brightness of the spider models from a longer distance, when they use green contrast vision rather than colour vision (Chittka 2001, Chittka and Raine 2006).

There was no significant difference in the amount of time honeybees spent within the patch (Fig. 9) or on the individual flower (Fig. 10) after making their choice. Preference for patches and individual flowers was thus solely determined by amount of visits and not by spending more time in a more favourable patch than in a less favourable one. This observation corresponds with Robertson and Klemash Maguire (2005), who found that *Misumena vatia* presence on a flower did influence the number of visitations by pollinators, but not the

duration of the visits. Even after failed spider attacks in the patch harbouring *Thomisus spectabilis*, honeybees often stayed within the patch and even visited the flower where previous danger was perceived a second time (personal observation). This observation does not correspond with the findings of Dukas and Morse (2005) where honeybees avoided patches where crab spiders were present. There are several possible explanations for the result observed in this experiment. Firstly, the crab spider species used in the experiment of Dukas and Morse (*Misumena vatia*) is UV-absorbing and does thus not attract honeybees. The attracting effect of UV-reflecting crab spiders could counteract the deterring effect of crab spider presence. Furthermore, *T. spectabilis* naturally occurs in Queensland, while the experiment was performed in Sydney, New South Wales. Honeybees visiting the experimental patches most likely had no previous experience with *T. spectabilis*. This does however not explain why they were often found to return to flowers where they had very recently experienced a failed spider attack.

Within-patch analyses

Within the patch containing white, UV-reflecting crab spider models, honeybees did not have a preference for either vacant flowers or flowers harbouring a spider model (Fig. 11). Within the patch containing yellow, UV-absorbing crab spider models, honeybees preferred to land on vacant flowers. It was expected that honeybees would prefer to land on flowers harbouring white crab spiders. The result is thus surprising. Honeybees seemed to be deterred by the presence of yellow spider models on the flowers once they were inside the patch. Possibly, honeybees are deterred by the presence of a foreign object on the flowers, but the attractive effect of the UV-reflecting white spider models counteracted this, resulting in a more equal visitation number of both vacant and occupied flowers. Dukas (2001) reported honeybees avoid flowers with immobile spiders. These were however not cryptic, unlike the yellow spiders used in this experiment.

Future research

Many questions remain after this research that could develop into future projects around this topic. During this experiment, there was little pollinator activity and only honeybees visited the experimental patches in sufficient numbers, making comparison with other pollinator species impossible. Repeating the experiment is essential for obtaining more data on both

honeybees and different pollinator species. White flowers and yellow *T. spectabilis* could be included as well for completeness of the experiment. Performing the experiment in the vicinity of a bee hive would ensure sufficient numbers of bees, but this would be contrary to the idea of the experiment to be performed in a more natural field setting with naturally occurring pollinators. An experiment using native bees could however be performed in a semi-natural setting to test the hypothesis of yellow crab spiders being more successful at capturing native bees than white crab spiders.

Apart from colour and brightness contrasts, several other factors determine flower choice by pollinators, such as flowers size and age. In natural settings, when flower age and size as well as ambient temperature, humidity and solar radiation cannot be controlled, it is harder to determine whether the effects observed during the experiment are only due to spider presence or also influenced by other components. It would be interesting to note flower size and flower age in future experiments to rule out the possibility of hidden variables.

It would also be interesting to further deepen the knowledge about the effect of colour contrasts caused by crab spiders on the attraction of pollinators using a different kind of contrast, for example, yellow UV-absorbing *Thomisus spectabilis* are often found on yellow flowers with UV-reflecting parts in nature (personal observation, Chittka 2001). It would be interesting to see what kind of effective this different type of contrast has on pollinator behaviour.

References

Briscoe, A.D. (2001) The evolution of colour vision in insects. *Annual Review of Entomology*, 46: 187-193

Chittka, L. (2001) Camouflage of predatory crab spiders on flowers and the colour perception of bees (Aranida: Thomisidae / Hymenoptera: Apidae). *Entomologia Generalis*, **25(3):** 181-187

Chittka, L. and Raine, N.E. (2006) Recognition of flowers by pollinators. *Current Opinion in Plant Biology*, 9(4): 428-435

Dukas, R. (2001) Effects of perceived danger on flower choice by bees. *Ecology letters*, 4: 327-333

Dukas, R. and Morse, D.H. (2003) Crab spiders affect flower visitation by bees. *Oikos* 101: 157-163

Dukas, R. and Morse, D.H. (2005) Crab spiders show mixed effects on flower-visiting bees and no effect on plant fitness components. *Ecoscience*, 12(2): 244-247

Heiling, A.M., Herberstein, M.E. and Chittka, L. (2003) Pollinator attraction: Crab-spiders manipulate flower signals. *Nature*, 421, 334

Heiling, A.M and Herberstein, M.E (2004a) Predator-prey coevolution: Australian native bees avoid their spider predators. *Proceedings of the Royal Society*, 271(4): 196-198

Heiling, A.M. and Herberstein, M.E. (2004b) Floral quality signals lure pollinators and their predators. *Annales Zoologici Fennici*, 41: 421-428

Heiling, A.M., Cheng, K., Herberstein, M.E. (2004) Exploitation of floral signals by crab spiders (*Thomisus spectabilis*, Thomisidae). *Behavioral Ecology*, 15(2): 321-326

Heiling, A.M., Chittka, L., Cheng, K. and Herberstein, M.E. (2005a) Colouration in crab spiders: substrate choice and prey attraction. *The Journal of Experimental Biology*, 208: 1785-1792

Heiling, A.M., Cheng, K., Chittka, L., Goeth, A. and Herberstein, M.E. (2005b) The role of UV in crab spider signals: effects on perception by prey and predators. *The Journal of Experimental Biology*, 208: 3925-3931

Heiling, A.M., Cheng, K. and Herberstein, M.E. (2006) Picking the right spot: crab spiders position themselves on flowers to maximize prey attraction. *Behaviour*, 143: 957-968

Herberstein, M.E., Heling, A.M. and Cheng, K. (2008) Evidence for UV-based sensory exploitation in Australian but not European crab spiders. *Evolutionary Ecology*, ISSN 1573-8477

Morse, D.H. (2007) Predator upon a flower: Life history and fitness in a crab spider. *Harvard University Press*, ISBN-13: 978-0-674-02480-9, 377pp

Lunau, K. Wacht, S. and Chittka, L. (1996) Colour choices of naïve bumble bees and their implications for colour perception. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 178(4): 477-489

Lunau, K. (2000) The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution*, 222(1-4): 89-111

Pollard, S.D. (1989) Constraints affecting partial prey consumption by a crab spider, *Diaea* sp. indet. (Araneae: Thomisidae). *Oecologia*, 81: 392-396

Robertson, I.C. and Klemash Maguire, D. (2005) Crab spiders deter insect visitations to slickpot peppergrass flowers. *OIKOS*, 109: 577-582

Théry, M. and Casas, J. (2002) Predator and prey views of spider camouflage. *Nature*, 415: 133

Wignall, A.E., Heiling, A.M., Cheng, K. and Herberstein, M.E. (2006) Flower symmetry preferences in honeybees and their crab spider predators. *Ethology*, 112(5): 510-518