

# Colour and shape preferences of *Apis cerana* (Java genotype) in Australia

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## Abstract

The biology of *Apis cerana* F., the Asian honey bee, is far less known than that of its sister species *Apis mellifera* L. The arrival of *A. cerana* in North Queensland has prompted the need to better understand the ecology and biology of this species in an invasive context. We evaluated the colour, shape and spontaneous landing preferences of free flying *A. cerana* using artificial shape of equal surface. *A. cerana* displayed a stable and marked preference toward yellow regardless of the season (wet or dry). However, for other colours, different preference patterns were observed depending on the season suggesting a learned preference. Bees had a strong preference for star shaped U.V. nectar guides regardless of the season. Conversely to *A. mellifera*, *A. cerana* appeared to minimise the perimeter surface ratio in its landing choice choosing circular over jagged surfaces. However, when tested using polygons and circle of same area and thus very similar perimeters the choice pattern showed no minimisation of perimeter/surface ratio. Surprisingly, bees had a clear preference towards odd number apex shapes and 3/4 of landings occurring on the heptagon, despite the rarity of such 7-lobed flowers in nature.

**Key words:** Asian honey bee, *Tetragonula* sp., bilateral symmetry, radial symmetry, flower traits.

## Introduction

The Asian honey bee (AsHB), *Apis cerana* F. (Hymenoptera Apidae) Java genotype, is a tropical bee species that recently breached quarantine in northern Australia (Koetz, 2013). It is classed as an invasive pest with the potential to detrimentally impact upon Australian bee-keeping and other agricultural industries. New research is investigating the behaviour of AsHB with the aim of developing AsHB-specific trapping stations to impede colony spread.

Interactions between flowering plants and their insect pollinators are the result of long co-evolutionary relationships that shape the attributes of both participants. Newly emerged honey bee foragers are known to exhibit spontaneous colour and shape preferences, even upon their maiden flight, a characteristic, which enables them to rapidly identify potential food sources and reduce their reliance on chance. Since the life span of honey bee foragers is typically in the realm of 6.3 days (Dukas, 2008), such spontaneous preferences are extremely beneficial for the survival of the hive.

Very little is known of the preference of AsHB in terms shape and colour preference, and a survey of the literature did not yield any results (see also Koetz, 2013). In contrast, the colour preferences of *Apis mellifera* L. have previously been investigated. In Europe, Giurfa *et al.* (1995) showed that *Apis mellifera ligustica* Spinola preferred violet over blue over yellow (violet>blue>yellow). In a similar European study *A. mellifera* bees were found to choose colours between 400-445 nm wavelengths, corresponding to the violet - indigo part of the spectrum. However, a study on the same species in Australia showed that the bees showed a significant preference for yellow over blue (Guez *et al.*, 2010; 2012). This suggest that colour preference in *A. mellifera* may have evolutionarily diverged after their in-

roduction in Australia, which is *a priori* unlikely, and/or may be a consequence of environmental factor such as the relative proportion of the colour of flower most often encounter by foragers in Europe and in Australia. Previous work have shown that *A. mellifera* prefer shape that are radially symmetrical versus bilaterally symmetrical, which would correspond to actinomorphic and zygomorphic flower respectively (Free, 1970; Wignall *et al.*, 2006).

The aims of the present study are to investigate the spontaneous colour (i.e. without experimental training) and shape preferences of AsHB found in the incursion site in Cairns. The overarching goal was to enable the design of an optimum baiting station for *A. cerana* and we thus investigated the colour preference of a *Tetragonula* sp. also present in Cairns to see if a simple colour choice may exclude them from a baiting station.

## Methods

All field experiments were performed in Cairns, a regional city in the far north of tropical Queensland, Australia. Food grade sucrose sugar was used for all experiment at a concentration of 60% (w/w), as this concentration had previously been determined as optimal for both AsHB and *Tetragonula* sp. during preliminary experiments. For our colour preference experiments we used white (Reflex Ultra White, Australian Paper), Quills Brights assorted (Quill Australia, <http://www.quill.com>) blue, yellow and green coloured paper placed around the feeders.

We used AsHB (Java genotype) workers introduced accidentally in 2007 in Australia (Koetz, 2013) from a feral hive found approximately 300 m from the experimental site, and *Tetragonula* sp. workers from natural hives around our experimental site. Workers of both

species were first trained to visit a colourless, Perspex, gravity fed 60% w/w sucrose feeding station that was elevated 30 cm from the ground by way of identical buckets. When a large number of foragers had learned to visit the feeding station site so that at least 50 foragers were simultaneously present, the experiment was initiated. Since we had previously observed that *A. mellifera* rapidly overcame AsHB at test feeders, any foraging *A. mellifera* bees were immediately removed and disposed of in order to avoid their further recruitment.

## Experimental procedure

### Colour preference

Foragers were first trained to visit a colourless, Perspex, gravity fed 60% w/w sucrose feeding station on a platform 30 cm from the ground (preliminary work demonstrated that it was the optimum sucrose solution when compared to 30, 40 and 50% w/w solution). When a large number of foragers had learned to visit the respective feeding station (i.e. at least 50 foragers present at the feeder simultaneously), the study was initiated.

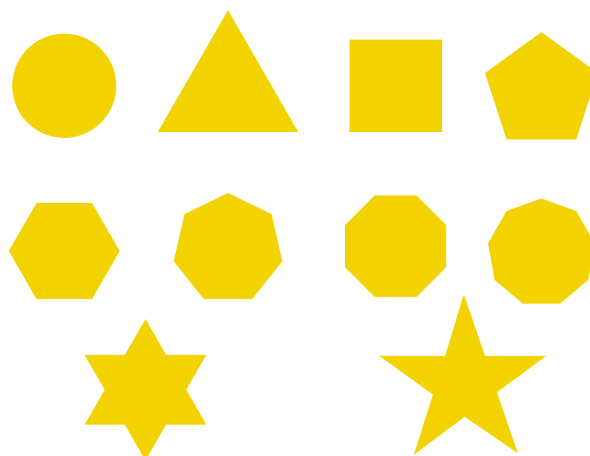
During all experiments, four test feeders were placed on each vertex of a square of 40 cm sides. In the case of three feeder experiments, the feeders were placed at the three vertex of an equilateral triangle with a 40 cm edge. This choice was based upon previous work by Huber *et al.* (1994), that found that *A. mellifera* foragers were able to successfully distinguish different targets if they were spaced by 40 cm.

The position of each feeder was pseudo-randomly changed at regular intervals (clockwise, until each feeder have occupied all possible position). Positional changes (two adjacent feeders were swapped before clockwise rotation until all feeders have occupied all possible position) were made so that each feeder had occupied each position for the same amount of time, and had been adjacent to all other possible feeder choices on at least four occasions.

Colour preference experiments were repeated during the wet and dry seasons to evaluate the stability of AsHB colour preferences, the mean yearly rainfall in Cairns is about 1982.3 mm (1981-2010) with 1709.6 mm of rain during the wet season (November to March). During the same period, the maximum temperature were 31.6 °C and 24.9 °C during the wet and dry season respectively while the minimum temperature were 22.5 °C and 17.1 °C.

### Forager landing

The number of foragers spontaneously attracted to each feeder was determined by setting up a test feeder 3-4 m from the training feeder site (a training feeder with bee foragers on it was moved to the experimental location just prior to the start of the experiment). This was to initially reduce the number of bees visiting the feeders so that they could be accurately counted. The number of bees foraging at each feeder was then counted at 30 seconds intervals over 30 minutes. The feeders were moved pseudo randomly every 2 minutes to account for possible position preferences.



**Figure 1.** Shape tested (not to scale).

### Statistical analysis

Data were analyzed using repeated measure ANOVA with no assumption of sphericity (Geisser-Greenhouse correction) after arcsine transformation in the case of ratio data. Where appropriate, these were followed by Newman-Keuls pairwise comparison.

### Shape preference

For all experiments we used yellow target placed horizontally. All target had the same surface area (figure 1). Since ultraviolet nectar guide have an influence on flower choice we tested bullseyes and star shaped nectar (nectar guide were painted using Rosco™ Vivid Fx UV special effects paint, bright white 6250). In the case of the choice between three possible shape, the shape where arranged on the apex of an equilateral triangle, and rotated pseudo randomly until each shape had occupied each possible position. In the last experiment, where 6 shapes were used, the position of each individual shape was randomized for each of the 3 repetitions.

### Forager landing

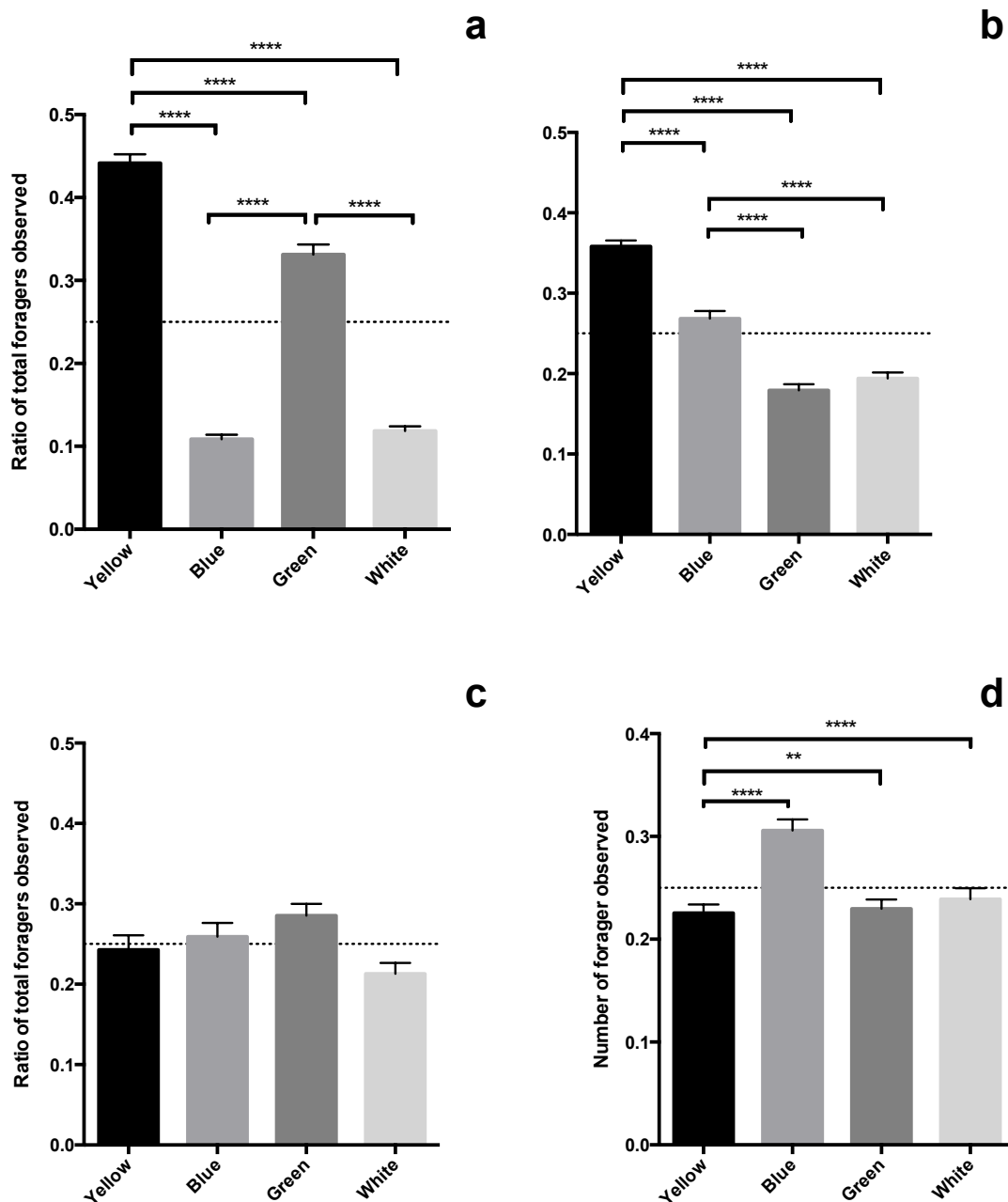
The number of foragers landing on each shape after removal of the training feeder was video recorded for 15 minutes for each shape relative position tested and later analysed.

### Statistical analysis

Data were analyzed using a  $\chi^2$  against expected choice if choice were random.

## Results

We first determined the “spontaneous” colour preference displayed by feral AsHB in the Cairn region in far north Queensland the result are presented figure 2a-b. The result displayed were obtained during the wet (figure 2a) and dry (figure 2b) seasons to evaluate the stability of AsHB colour preferences. The first notable result is that relative colour preference for blue, green and white is not stable across time with green being preferred in comparison of both blue and white during the

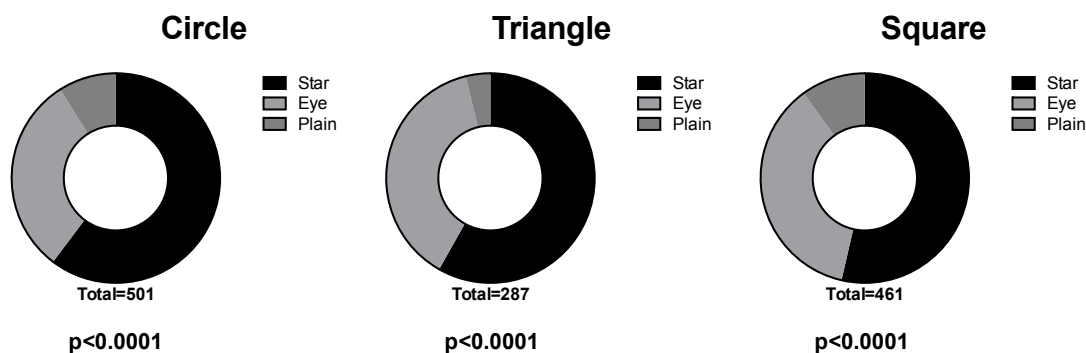


**Figure 2.** Colour preference of *A. cerana* in wet (a) and dry season (b); colour preference of *Tetragonula* sp. in wet (c) and dry season (d). \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ .

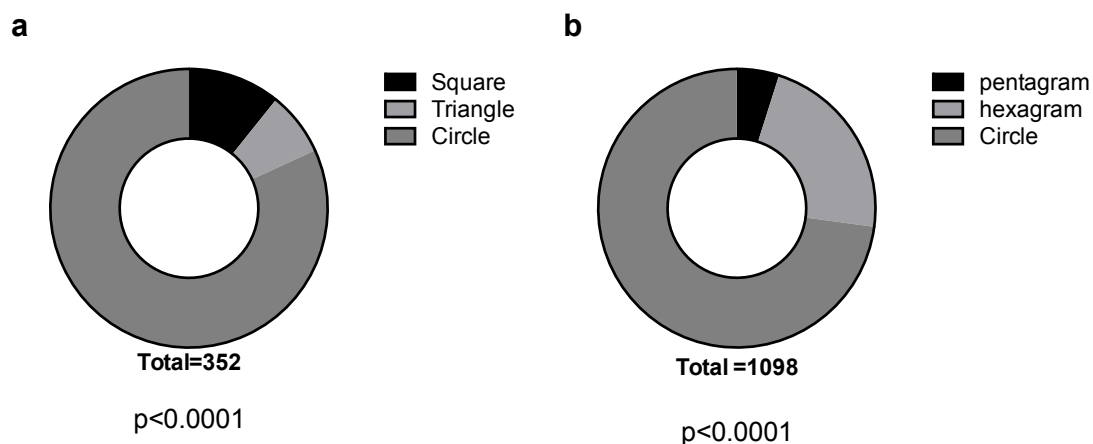
wet season (figure 2a) whereas during the dry season blue is preferred in comparison of both the green and white colour (figure 2b). However, a clear preference for yellow is clear for both test point suggesting an enduring and stable preference for yellow (compare figure 2a-b).

However, as stated in introduction, one needs to consider *Tetragonula* sp. Thus we also investigated colour preference of *Tetragonula* forager using the same colour choice used for AsHB. The results are displayed figure 2c-d. It is clear that during the wet season (figure 2c) *Tetragonula* forager display no preference for the colour tested and seems to choose at near random. However the ANOVA result is marginally significant:  $F(2.515, 150.9) = 2.589$ ,  $p = 0.0653$ , and if pairwise comparison (Newman-Keuls) are performed a significant difference

between green and white exist ( $p < 0.05$ ). In contrast during the dry season a clear preference for blue is evident:  $F(2.608, 156.5) = 11.28$ ,  $p < 0.0001$ . This suggests that colour preference in *Tetragonula* sp. is not stable across time, suggesting that preferences displayed are acquired. However, one could argue that if this was the case colour preference of AsHB and *Tetragonula* sp. in the same environment should be similar since both species should be exposed to the same flowers. Nevertheless, the foraging range of *Tetragonula* sp. is far smaller than the one of AsHB (few hundred metre compared to few kilometres), suggesting that although the environment of *Tetragonula* could be at certain point of time dominated by blue flower it may not be true for AsHB nesting around the same location.



**Figure 3.** Nectar guide preference in function of the shape of the target in *A. cerana*.



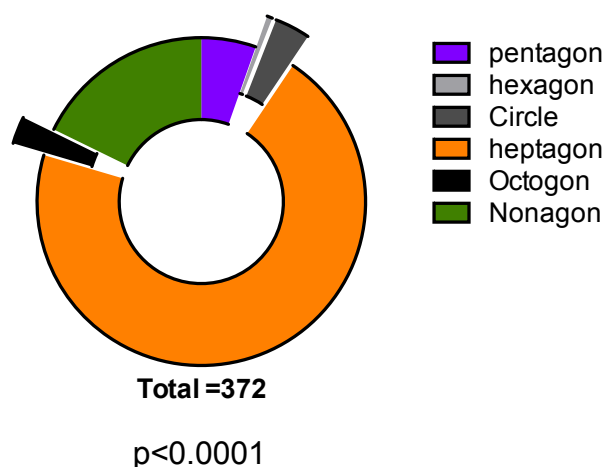
**Figure 4.** Shape preference in *A. cerana*. **(a)** circle versus triangle and square; **(b)** circle versus pentagram and hexagram.

The nectar guide (bulleyes or star shaped) preference of AsHB using three arbitrary yellow shape (square, circle and equilateral triangle) is shown figure 3 (yellow was chosen since AsHB demonstrated a stable preference for this colour). The AsHB shows a clear preference for shape that include nectar guides and prefers star shaped nectar guide regardless of the shape investigated (circle  $\chi^2 = 199.3$ ,  $df = 2$ ,  $p < 0.0001$ ; triangle  $\chi^2 = 130$ ,  $df = 2$ ,  $p < 0.0001$ ; square  $\chi^2 = 133.5$ ,  $df = 2$ ,  $p < 0.0001$ ). Figure 4a demonstrates surprisingly a strong preference for the circle vs triangle and square shape (all with star nectar guides), ( $\chi^2 = 373$ ,  $df = 2$ ,  $p < 0.0001$ ).

Following on this surprising preference for the circle shape, we decided to pitch against it to radially symmetrical shape showing increased edging (a pentagram and a hexagram), once again and surprisingly AsHB show a preference for the circle shape ( $\chi^2 = 821.3$ ,  $df = 2$ ,  $p < 0.0001$ ) (figure 4b).

This preference, for a circular shape, is surprising unless we imagine that flower visited by AsHB in the Cairns region have a small edge (perimeter) to surface ratio. Furthermore, this result is clearly unexpected given that *A. mellifera* preference are the antipode of the one expressed by *A. cerana* (Free, 1970). Figure 5 show the relative preference of AsHB for circle, pentagon,

hexagon, heptagon, octagon, and nonagon. The AsHB show clear preference toward heptagon shape ( $\chi^2 = 807.8$ ,  $df = 5$ ,  $p < 0.0001$ ), interestingly AsHB seems to prefer shape with an odd number of apex, which represent more than 93% of all choice made.



**Figure 5.** *A. cerana* shape preference in function of the number of apex present (in colour odd number of apex).

## Discussion

Is this preference for yellow acquired or innate? it is difficult to disambiguate. However, the fact that a preference for yellow in *A. mellifera* have been previously reported in Australia (Guez *et al.*, 2010; 2012), whereas blue or violet preference have been describe for the same species in Europe (Giurfa *et al.*, 1995, but see Celli *et al.*, 1987) however a review of the literature cited in Giurfa *et al.*, 1995, suggests that a yellow alternative was never provided during these works. Admitting that a blue preference can be confirmed in Europe, it suggests that it is most likely acquired due to a large proportion of yellow flowers in Australia. Nonetheless, this result suggests that the use of yellow feeder may be a good choice if one is attempting to attract AsHB to it despite the fact that they may also attract *A. mellifera* forager. However, the difference of size between *A. mellifera* and *A. cerana* forager should make it easy to exclude the former from accessing the feeders.

In term of shape preference, our first comparison revealed a preference for shape that included U.V. nectar guides: bulleyes or star shaped, over shape that did not have them. Furthermore, bee displayed a marked preference for star shaped nectar guide, thus for all our following experiment we included star shaped nectar guides. The possible importance of nectar guide has been suggested in early work with *A. mellifera* (Free, 1970) and more recently for bumblebees (Leonard *et al.*, 2013). Surprisingly AsHB displayed a preference for round target over square or triangular ones. This may appear surprising simply because flowers are rarely circular. Nonetheless, it has been shown that for target of equal area, circular ones are perceived at a longer distance (Ne'eman and Kevan, 2001), which may explain the landing choice of the bees in this experiment. Regardless another possibly important feature of this comparison is that it could appear that the choice may be based on the perimeter length of each shape since the perimeter of the triangle>square>circle, suggesting that for a given area bees choose the smaller perimeter shape.

Because flowers are rarely circular, we compared the choice of the bee using again the circle but this time star shape (pentagram and hexagram) of the same surface area that may better represent the shape of flower. We found once again that the circle shape was chosen more often. This result may again be explained by the fact that circular shapes are perceived from a longer distance. However, once again the results can also be explained in term of perimeter length with pentagram>hexagram>circle, suggesting once again that for a given surface area bees choose the smaller perimeter shape.

Thus we compared shape of the same surface area, which were sharing very similar perimeter (circle, pentagon, hexagon, heptagon, octagon, and nonagon, mean perimeter =  $21.13 \pm 0.2154$ ). If the bees were choosing the target based solely on their perimeter, one would expect a nearly uniform choice or if the bee are extremely accurate a choice of the circular target which again has the smaller perimeter given the area. However, the bee chose the heptagon shape (70.16% of the

time). Furthermore, if we take into account all the shape with bilateral symmetry the choice raise over 82% of the time (pentagon 5.376%, heptagon 70.16% and nonagon 17.74%). Furthermore, if we ignore the main choice for the heptagon, and pull together the shape with bilateral symmetry (pentagon and nonagon) and the ones radially symmetrical (hexagon and octagon) we find that AsHB prefer clearly bilateral symmetry (binomial test,  $p < 0.0001$ ) in complete opposition of *A. mellifera* that display a clear preference for radial symmetry (Free, 1970) has long as their perimeter is close to the one of a circle of same surface. It would appear that AsHB display a preference for shape with bilateral over radial symmetry by opposition to *A. mellifera* radial symmetry preference. The clear difference in preference of *A. mellifera* and its sister specie *A. cerana* may have been the consequence of different selection pressure.

## Conclusion

Our study reveals that *A. cerana* in the Cairns region display a stable preference for yellow, for shape that include star like U.V. nectar guide and a strong preference for heptagon shape and more generally for radial symmetry has long as the shape perimeter is close to the one of a circle of same surface. The underlying reason for heptagonal shape preference is unclear since it would appear that 7-lobed flowers are quite rare compared to penta and hexa-lobed one. Nonetheless, based on our work, the best visually attractive *A. cerana* baiting station should display yellow heptagon with star like U.V. nectar guides.

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