

## Reduction of Visitation Rates by Honeybees (*Apis mellifera*) to Individual Inflorescences of Lavender (*Lavandula stoechas*) upon Removal of Coloured Accessory Bracts (Hymenoptera: Apidae)

ANDREW D HIGGINSON, FRANCIS S GILBERT, TOM READER & CHRISTOPHER J BARNARD

Received: 2006-06-02/2006-06-30

Accepted: 2006-09-09

HIGGINSON A D, GILBERT F S, READER T & BARNARD C J [Anim Behav Ecol; Univ Nottingham, NG7 2RD]: **Reduction of Visitation Rates by Honeybees (*Apis mellifera*) to Individual Inflorescences of Lavender (*Lavandula stoechas*) upon Removal of Coloured Accessory Bracts (Hymenoptera: Apidae)** – Entomol Gener **29**(2/4): 165–178; Stuttgart 2007-01. – – – [Article]

Honeybees (*Apis mellifera* Linnaeus 1758) choosing between inflorescences of *Lavandula stoechas* appear to use a two-stage decision process based on different morphological criteria at each stage. The first stage, whether to closely inspect inflorescences, is based on display size. *Lavandula* inflorescences have large showy terminal bracts at the apex which increase the apparent display size. However, crab spiders often conceal themselves by closing the bracts around themselves using silk. Two manipulation experiments were carried out on the effects of the bracts on visitation rates to individual inflorescences. In the first, inflorescences from which bracts were removed received a reduced rate of inspection. In the second, inflorescences in which bracts were stuck together, mimicking the spider behaviour, experienced the same number of inspections as controls. Hence, the bracts did play a role in attracting honeybees to inflorescences, perhaps because a lack of bracts is usually seen only in very young or very old inflorescences, neither of which produce any nectar. However, honeybees did not avoid the closed bracts (assumed to provide an indirect cue of spider presence), probably because spiders often move between inflorescences, thus making closed bracts unreliable as a cue to predation risk.

**Key words:** *Apis mellifera* (Linnaeus 1758) – crab spider – display size – pollination – predation risk – scent marking

HIGGINSON A D, GILBERT F S, READER T & BARNARD C J [Ethol Ökol Tiere; Univ Nottingham, NG7 2RD]: **Senkung der Besuchsraten von Honigbienen (*Apis mellifera*) an einzelnen Blütenständen des Lavendels (*Lavandula stoechas*) bei Entfernung farbiger Tragblätter (Hymenoptera: Apidae)**. – Entomol Gener **29**(2/4): 165–178; Stuttgart 2007-01. – – – [Abhandlung]

Honigbienen (*Apis mellifera* Linnaeus 1758) scheinen unter Blütenständen von *Lavandula stoechas* nach einem zweistufigen Entscheidungsprozeß zu wählen, und zwar auf der Basis jeweils unterschiedlicher morphologischer Kriterien. Die erste Entscheidung, ob der Blütenstand genau inspiziert werden soll, basiert auf seiner scheinbaren Größe: *Lavandula*-Blütenstände besitzen große auffällige Tragblätter an der Spitze, welche die Blütenstände größer erscheinen lassen. Häufig verstecken sich Krabbenspinnen im Blütenstand, indem sie sich mittels Seide in diese Tragblätter einwickeln. In zwei Manipulationsexperimenten wurde der Einfluß der Tragblätter auf die Besuchsraten für einzelne Blütenstände untersucht. Im ersten Versuch wurden Blütenstände, deren Tragblätter entfernt worden waren, weniger häufig besucht.

Im zweiten Versuch wurden Blütenstände, deren Tragblätter zusammengeklebt worden waren, um das Verhalten der Spinnen nachzuahmen, nicht seltener inspiziert als die Kontrollblüten. Daraus ergibt sich, daß die Tragblätter eine Rolle bei der Anziehung der Honigbienen spielten, vielleicht weil Tragblätter in der Regel nur bei sehr jungen oder sehr alten Blütenständen fehlen, die beide keinen Nektar produzieren. Honigbienen haben die zugeklebten Tragblätter (von denen angenommen worden war, sie würden als indirekter Hinweis auf die Anwesenheit von Krabbspinnen dienen) jedoch nicht vermieden, vermutlich weil Spinnen sich oft hin und her bewegen unter Blütenständen, so daß die geschlossenen Tragblätter nur unzuverlässige Hinweise auf das Prädationsrisiko darstellen.

**Schlüsselbegriffe:** *Apis mellifera* (Linnaeus 1758) – Bestäubung – Blütengröße – Duftmarkierung – Krabbspinnen – Prädationsrisiko

## 1 Introduction

Models of optimal foraging which seek to explain patterns of behaviour in insects foraging for nectar and pollen often ignore the effects of predators [MORSE 1986a, LIMA 1998, DUKAS 2001b], largely on the assumption that predation is too rare to exert strong selection [PYKE 1979, SCHMID-HEMPEL 1991, SCHMALHOFFER 2001]. However, if honeybees are adapted to maximise their total lifetime energy input to the hive, we might expect them to take some steps towards prolonging their foraging lifespan. One predation pressure is exerted by crab spiders (Arachnidae: Thomisidae) which are ambush predators that pounce on flower visitors that land on nectar sources [MORSE 1986b]. Crab spiders can camouflage themselves on flowers [CHITTKA 2001] and even make flowers or inflorescences they are on more attractive to foragers [HEILING, HERBERSTEIN & CHITTKA 2003]. There is also some evidence that bees avoid foraging in areas of high spider density [DUKAS & MORSE 2003], and avoid signs of spider presence [DUKAS 2001a, READER, HIGGINSON, GILBERT et al 2006], indicating that they take some measures to safeguard their future survival.

Recent work by the authors has concentrated on honeybees foraging on the Mediterranean shrub lavender (*Lavandula stoechas*) [HIGGINSON & BARNARD 2004, HIGGINSON, GILBERT & BARNARD 2006, READER et al 2006]. *Lavandula* flowers grow as inflorescences, which usually have a set of 1–6 colourful sterile bracts at the apex. Previous work on choice of *Lavandula* inflorescences by honeybees has produced a contradictory picture of the effects of the terminal bracts. In previous work inflorescences have been categorised according to observed bee responses to them. Inflorescences that bees alighted on were categorised as accepted. Before alighting, bees orientated towards and hovered close to inflorescences, appearing to inspect them closely, probably to assess whether to alight. Inflorescences that bees inspected but on which they did not alight were called rejected. Bees passed close (< 3cm) to many other inflorescences that they were assumed to have detected but did not pause to inspect; these inflorescences were categorised as ignored. Of course, it is possible that ignored inflorescences were, in some cases, simply not detected.

While bees are initially attracted to inspect inflorescences with larger, more numerous bracts, they appear to accept inflorescences with smaller bracts [DUFFIEL, GIBSON, GILHOOLY et al 1993, GONZALEZ, ROWE, WEEKS et al 1995], and at this stage the decision appears to be based on the number of flowers and inflorescence age [HIGGINSON et al 2006]. It may be that bees do not use the spatial distribution of flowers on the inflorescence, or absolute number of flowers, but they appear to use some correlate of age and number of flowers, which could also be colour or patterns of colour [GIURFA & VOROBYEV 1997, SPAETHE, TAUTZ & CHITTKA 2001, DYER & CHITTKA 2004] to choose the more productive inflorescences. The bracts grow as the inflorescence ages, but stop growing by the time the number of flowers begins to decline [HIGGINSON et al 2006]. Thus bract length is not related in a simple way to the number of flowers, nor to the nectar secretion rate, which may confound the bees' learned responses to bract size and number. [HERRERA 1987] experimentally removed all the bracts from *L. stoechas* bushes and observed a clear reduction (23%) in visitation rate by bees. When a bush was spatially split in half, and all the bracts were removed from one half, that half experienced 41% fewer visits than the control half.

Honeybees are more likely to reject *Lavandula* inflorescences that contain spiders [READER et al 2006] and several cues could potentially indicate the presence of a spider. The bee may be able to detect the spider directly by smell or sight. The latter is unlikely since the spiders are usually not visible except from directly above the inflorescence, and even then not always because the bracts are often joined together at the top by the spider. Alternatively, the bee may simply avoid inflorescences where there is a high probability of spider occupancy, i.e. those with at least four large bracts. Spiders usually conceal themselves within the terminal bracts, tying them together with silk (A D HIGGINSON, pers obs). Spiders also select among inflorescences based on the number and size of bracts because they require a certain amount of bract material in which to conceal themselves [READER et al 2006]. Therefore, bees may learn that inflorescences that have at least four large bracts may be particularly likely to contain spiders, especially if the bracts are closed.

In order to investigate the role of bracts in bee choice, two experiments were carried out where visits by bees to marked inflorescences were recorded before and after manipulation of the bracts. In the first experiment, the treatment was removal of the bracts. As HERRERA [1987] showed that removal of bracts reduces visitation rates to bushes or half-bushes, the aim of the first experiment was to assess whether such an effect was detectable at the level of visitation rates to individual inflorescences, and whether bees were less likely to inspect or accept manipulated inflorescences. It was predicted that removal of bracts should reduce the number of bees inspecting inflorescences. However, if the bracts that were removed were closed and large enough to conceal a spider, bees may be more likely to accept these inflorescences when the bracts were removed. In the second experiment, the aim was to assess whether bees avoid inflorescences with closed bracts. Therefore, the bracts of some inflorescences were stuck together as though by a spider and it was predicted that bees would be less likely to accept these inflorescences.

Honeybees sometimes use scent marks in order to avoid visiting inflorescences that have recently been depleted of nectar [GIURFA & NUNEZ 1992, WILLIAMS 1998, GOULSON, CHAPMAN & HUGHES 2001]. The role of scent marks has not been studied in the current system, and it is possible that scent marks of previous visiting bees affects the decision whether to visit a *Lavandula* inflorescence. If bees were using scent marking, the probability that a bee visits an inflorescence should be lower if it has recently been visited.

## 2 Material and methods

Experiment I was carried out at the Quinta de São Pedro research station near Lisbon in Portugal (N 38° 33.67' W 009° 11.34') in April 2004. Experiment II was conducted in the nearby Paisagem Protegida da Arriba Fossil da Costa da Caparica in April 2005. At both sites large numbers of lavender shrubs containing many thousands of inflorescences were heavily exploited by honeybees.

All selected inflorescences were numbered with a small piece of masking tape, the day before the observations started, so that if the labels affected visitation, the foragers would have become habituated to them by the time of the experiment. On the days of manipulations, all inflorescences were measured for morphological characteristics previously shown to affect bees' decisions: length of inflorescence, length of longest bract, number of bracts, number of flowers and age of inflorescence. The age of inflorescence was recorded as the generation of the flowers (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) assessed by the spatial arrangement of the flowers, since the flowers of each generation are distinctively distributed around the inflorescence [HIGGINSON et al 2006]. The position of the bracts relative to one another was also noted. If they were together and enclosed a space large enough to conceal a spider they were classified as closed, otherwise they were open.

All decisions made by observed bees were classified into three groups following earlier work [DUFFIELD et al 1993, GONZALEZ et al 1995, HIGGINSON & BARNARD 2004] based on the bees' responses to inflorescences:

**Accepted inflorescences** were those on which a bee alighted and probed one or more flowers.

**Rejected inflorescences** were those that appeared to be inspected by a bee (and sometimes touched by the antennae or legs) in a brief hovering flight, but on which the bee did not alight.

**Ignored inflorescences** were those that a bee approached (within 3cm) but at which it did not visibly pause or make any physical contact. Such inflorescences may have been detected but ignored, or not detected, by the bee.

**Accepted and rejected inflorescences** will be referred to collectively as *inspected* inflorescences.

Bee decisions were recorded by a single observer in Experiment I, but two observers in Experiment II. Before behavioural recordings started, trial observations were carried out where both observers independently recorded decisions made by bees to check that classification of decisions were consistent across observers. The inspection behaviour is so distinctive that all decisions were categorised the same by both observers.

## 2.1 Experiment I: Removal of bracts

Four patches each of 100 inflorescences were chosen arbitrarily in a large stand of *Lavandula* of many thousands of inflorescences. Each patch was a section of a bush that was at least one metre from the bushes containing other designated patches. All 400 inflorescences were labelled with a small piece of numbered masking tape around the stem. For three days before and three days after manipulation, bees were observed between 1000 and 1800 h (GMT) foraging in the patches. Patches were observed in rotation for 20 minutes at a time, so approximately one quarter (2 hours per patch per day) of the activity at each patch was recorded. Most of the time there was only one bee in the patch at any one time. However, during busy periods there was more than one bee foraging in a patch, so a few visits may have been unrecorded some of the time. On the day of manipulation, 50 inflorescences were selected randomly on each patch and their bracts were removed: the other 50 inflorescences per patch were left as controls. It was expected that manipulated inflorescences, but not control inflorescences, would experience a reduction in the number of inspections.

## 2.2 Experiment II: Closure of bracts

Three patches were chosen arbitrarily in a large stand of *Lavandula* of, again, many thousands of inflorescences. Each patch was a section of a bush that was at least one metre from the bushes containing other designated patches. Within each patch, 40 inflorescences that had four or more bracts were marked with numbered masking tape. For four days before and two days after manipulation, bees were observed between 1000 and 1600 h (GMT) foraging in the patches. Patches were observed in rotation for 20 min at a time, so approximately one third (2 hours per patch per day) of the activity was recorded. The experimental manipulation in this case involved gluing (Opalithplättchen; E. H. Thorne [Beehives] Ltd, U.K.) the bracts together at the top (so they were closed) to imitate the spiders' behaviour. It was possible that the bees would respond to the glue, so controls for the presence of glue per se were included. As Experiment II was carried out in a different year and location, it was important to replicate the results of Experiment I, along with a control where bracts were removed and glue was added. Since closure of bracts may reduce the visible surface area of bracts, a control was also needed that reduced the area without closing the bracts. Hence, there were four treatments of 30 randomly selected inflorescences each that were randomly distributed among the three patches: (a) Control, otherwise unmanipulated bracts with glue added, (b) All bracts removed, glue added to stem below inflorescence to replicate the results of experiment one with glue, (c) Half bract material removed to reduce the visible surface area, glue added to other half, (d) Bracts stuck together at the top to close them.

It was expected that, if bracts attract bees to inflorescences, Treatment **b** inflorescences would experience a reduction in the number of inspections, and Treatment **c** inflorescences would experience a smaller reduction in the number of inspections. Furthermore, it was expected that, if bees avoid closed bracts, there would be a difference between Treatments **c** and **d** in the change of the number of inspections or the probability of acceptance.

### 2.3 Data processing and analysis

The data consisted of morphological data for all marked inflorescences, and the decisions by bees at numbered inflorescences. In Experiment 1, 2133 decisions were recorded (5.3 per inflorescence) and, in Experiment 2, 1147 decisions were recorded (9.6 per inflorescence). The spreadsheets were cross-referenced in Microsoft Excel by a VisualBasic macro resulting in the total numbers of acceptances, rejections and ignores before and after the manipulations for each inflorescence. In addition, in Experiment 2, the total number of acceptances, rejections and ignores previous to each decision within each 20 min period were summed in order to see whether there was any effect of recent visitation on the probability of accepting a given inflorescence.

Morphological data were analysed in SPSS (SPSS, Inc.), but all analyses of the number of visits to inflorescences were carried out in R (Free Software Foundation) using Poisson errors. Other data were non-normal and non-Poisson so non-parametric tests were used: in the text, 'KW<sub>i</sub>' implies the test statistic of the Kruskal-Wallis test (distributed as  $\chi^2$ ) with *i* degrees of freedom, and 'U' implies the large-sample statistic of a Mann-Whitney U test.

## 3 Results

### 3.1 Experiment I

Patches did not differ in the number of flowers on inflorescences (KW<sub>3</sub> = 2.21, *P* = 0.53), or age of inflorescences (KW<sub>3</sub> = 1.96, *P* = 0.58), but did differ in the length (KW<sub>3</sub> = 25.6, *P* < 0.001) and number of bracts (KW<sub>3</sub> = 28.4, *P* < 0.001) and inflorescence length (KW<sub>3</sub> = 19.29, *P* < 0.001). Inflorescences in patch one had fewer smaller bracts, and patch four inflorescences were shorter than those in other patches. There was no significant difference in any of the morphological measures between the control and experimental inflorescences (U tests, *p* > 0.37 in all cases).

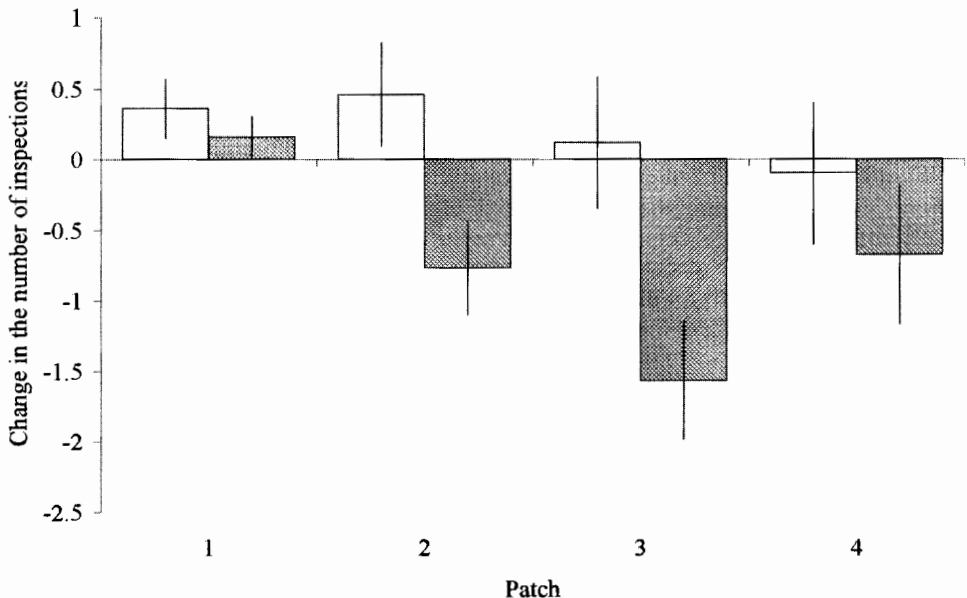
Day number (starting with the first day of observations as Day 1) was entered into the analysis to check that there was not an overall decrease in visitation rate over the course of the experiment. While there were differences between patches, the visitation rate to each did not decline over the course of the experiment ( $F_{3,376} = 0.966$ , *P* = 0.409), as may have been expected to happen due to the removal of some the bracts in the patches.

Principal component analysis (PCA) was performed on the morphological measures of all inflorescences to derive independent axes of variation among inflorescences. The PCA yielded five components of which the first two together accounted for 70% of the variance and reflected loadings similar to the PCs used in previous studies [DUFFIELD et al 1993, GONZALEZ et al 1995, HIGGINSON & BARNARD 2004]. The first (explaining 38.97% of the variance) mainly reflected the length of the inflorescence and the number and length of the bracts, and the second (explaining 31.29%) contrasted the number of flowers (positive) and the age of the inflorescence (negative). The first therefore reflected overall size (hereafter *Size*), and the second reflected morphological characteristics that closely predict the amount of nectar available (hereafter *Quality*) [HIGGINSON et al 2006].

Using multiple regression analysis, it was found that the number of acceptances per inflorescence before the manipulation was positively related to *Quality* of inflorescences ( $G_{1,389} = 5.626, P < 0.001$ ) but not their *Size* ( $G_{1,389} = 1.118, P = 0.264$ ). However, the total number of inspections (acceptances + rejections) was related to both *Size* ( $G_{1,389} = 2.315, P = 0.021$ ) and *Quality* ( $G_{1,389} = 4.205, P < 0.001$ ), supporting the hypothesis that bees inspect inflorescences on the basis of display size but accept based on number of flowers and age [HIGGINSON & BARNARD 2004, HIGGINSON et al 2006]. After the manipulation, the number of inspections of manipulated inflorescences was not related to the *Size* ( $G_{1,191} = 1.228, P = 0.221$ ), but only to the *Quality* ( $G_{1,191} = 4.404, P < 0.001$ ), since the bracts had been removed so the size had been reduced.

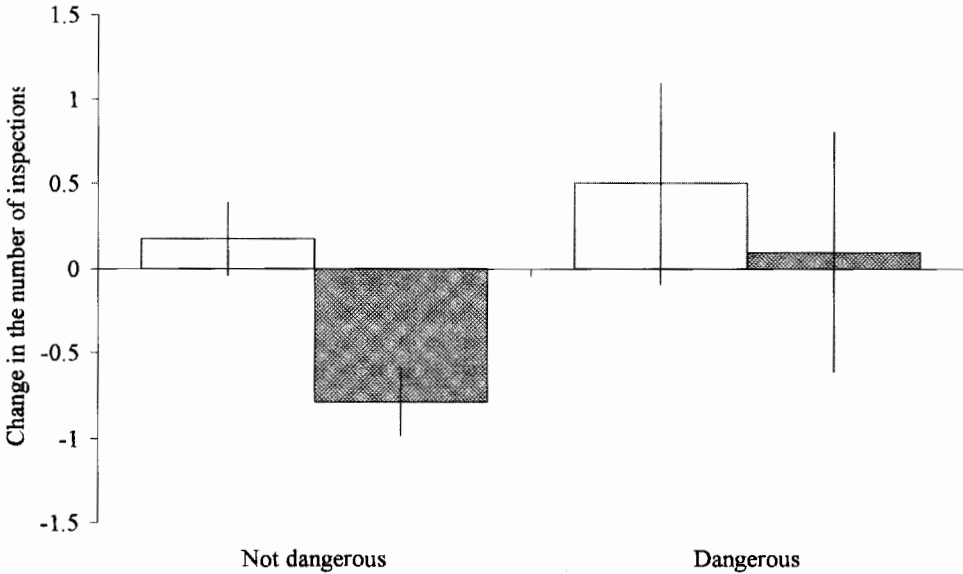
Two-way ANOVA with treatment and patch as factors showed that the change in the number of inspections was affected by the removal of bracts ( $F_{1,382} = 11.649, P = 0.001$ ): inflorescences that had their bracts removed were inspected less (a 25% decrease), and there was either no change or a slight increase for the controls. There was a significant difference between patches ( $F_{3,382} = 3.047, P = 0.029$ ), but no interaction between patch and treatment ( $F_{3,382} = 1.538, P = 0.204$ ), despite there being an increase of inspections of manipulated inflorescences on patch one (Fig 1). The same analysis on each decision showed that the change in number of acceptances was different between control and manipulated inflorescences ( $F_{1,382} = 4.853, P = 0.028$ ), as was the change in the number of rejections ( $F_{1,382} = 7.668, P = 0.006$ ), in that manipulated inflorescences experienced a fall in the number of acceptances and rejections (19%, 34% respectively) but controls did not.

Almost all spiders (91%) were found on inflorescences that had four or more bracts [HIGGINSON 2005, READER et al 2006], and pulled the bracts closed when they were waiting in ambush.



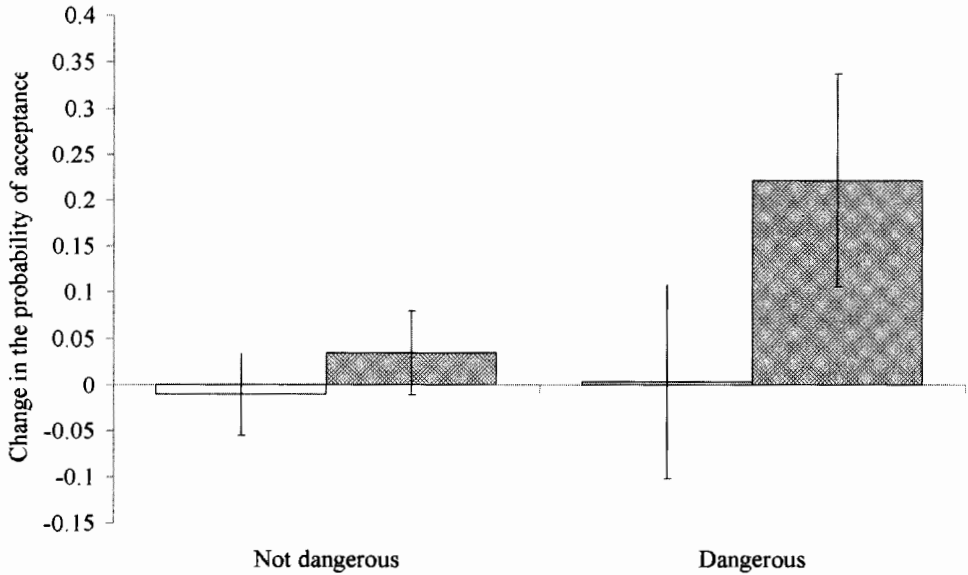
**Fig 1:** The mean (+1 SE) change in the number of inspections by honeybee workers (*Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] per lavender (*Lavendula stoechas* L) inflorescence for the control (open bars) and treated (hatched bars) inflorescences for each patch. Removal of the bracts caused a decrease in the number of inspections.

To test whether the position of the bracts (*open* or *closed*) had an effect on bee behaviour, an inflorescence was said to be *dangerous* if it had more than four bracts and they were *closed*. After manipulation the number of inspections of inflorescences with closed bracts which were removed did not decrease, unlike the number of inspections of all other manipulated inflorescences (**Fig 2**). Two-way ANOVA of the change in the number of inspections against the two factors of danger and treatment showed that this interaction was not significant ( $F_{3,388} = 0.366$ ,  $P = 0.546$ ), which was perhaps because there were only 39 *dangerous* inflorescences (compared to 353 others).



**Fig 2:** The mean (+1 SE) change in the number of inspections by honeybee workers (*Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] for lavender (*Lavendula stoechas* L) inflorescences that were (dangerous) or were not (not dangerous) able to conceal a spider for control (open bars) and treated (hatched bars) inflorescences.

Since the inspection process may involve an attempt to detect spiders, the risk of predation may affect the probability of acceptance once the inflorescence had been inspected, rather than the number of inspections. The change in the probability of acceptance was calculated as the difference between before and after the manipulation in the proportion of inspections that were acceptances. The change in the probability of acceptance suggested an interaction between predation risk and treatment, as only dangerous inflorescences whose bracts were removed experienced an increase after the manipulation (**Fig 3**). Hence, whilst there was no difference between non-dangerous control and manipulated inflorescences in the change in the probability of acceptance ( $U = 6687.5$ ,  $N_1 = 125$ ,  $N_2 = 111$ ,  $P = 0.631$ ), there was a difference between control and manipulated dangerous inflorescences, which was significant at the 1% level despite the small sample sizes ( $U = 75$ ,  $N_1 = 14$ ,  $N_2 = 17$ ,  $P = 0.077$ ). The probability of acceptance of inflorescences that were likely to contain a spider increased after the removal of bracts.



**Fig 3:** The mean (+1 SE) change in the probability of acceptance by honeybee workers, *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] for lavender (*Lavandula stoechas* L) inflorescences that were (dangerous) or were not (not dangerous) able to conceal a spider for control (open bars) and treated (hatched bars) inflorescences.

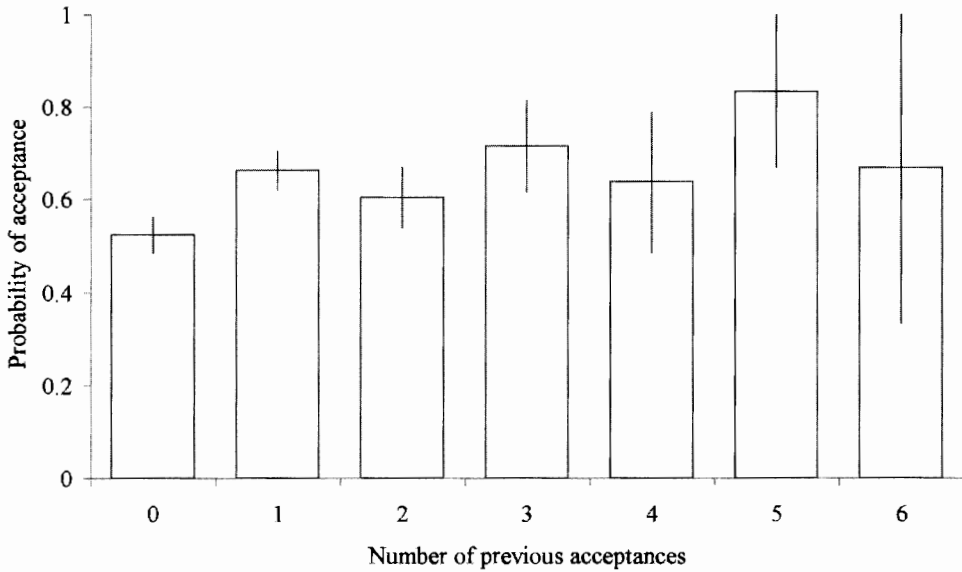
### 3.2 Experiment 2

Inflorescences in each treatment group did not differ in any of the morphological measures taken ( $KW_3 < 5.473$ ,  $P > 0.14$ ). There were no differences between treatment groups before the application of treatments in the number of acceptances, rejections and ignores per inflorescence ( $KW_3 < 5.442$ ,  $P > 0.142$ ).

In order to investigate whether the number of previous decisions affected whether an inflorescence was inspected, or whether it was accepted after inspection (binary variables), binary logistic regression was performed entering the total number of acceptances, rejections and ignores previous to each decision in a twenty-minute period and all morphological measures of inflorescences. Only the second half of decisions within each twenty-minute period before the application of treatments were used as the response variables so there would be no effect of treatments and to reduce inaccuracy in visitation observations due to the time since the start of the observation period. The probability of inspecting an inflorescence was not affected by the previous number of acceptances ( $G_{1,381} = 1.728$ ,  $P = 0.084$ ), rejections ( $G_{1,381} = 1.73$ ,  $P = 0.084$ ), or ignores ( $G_{1,381} = 0.244$ ,  $P = 0.807$ ), but only by the number of flowers ( $G_{1,381} = 3.467$ ,  $P < 0.001$ ). Similarly, the probability of accepting an inflorescence (once it had been inspected) was not affected by the previous number of acceptances ( $G_{1,343} = -0.164$ ,  $P = 0.87$ , **Fig 4**), rejections ( $G_{1,343} = 0.038$ ,  $P = 0.97$ ), or ignores ( $G_{1,343} = -0.426$ ,  $P = 0.67$ ), but only by the number of flowers ( $G_{1,343} = 4.754$ ,  $P < 0.001$ ), suggesting that previous acceptances, and hence scent marks, play little role in foraging on *Lavandula*.

The total number of inspections and ignores was much lower after the manipulation (inspected before: 666, inspected after: 307, ignored before: 106, ignored after: 68), probably as the nectar production of inflorescences deteriorated overall.

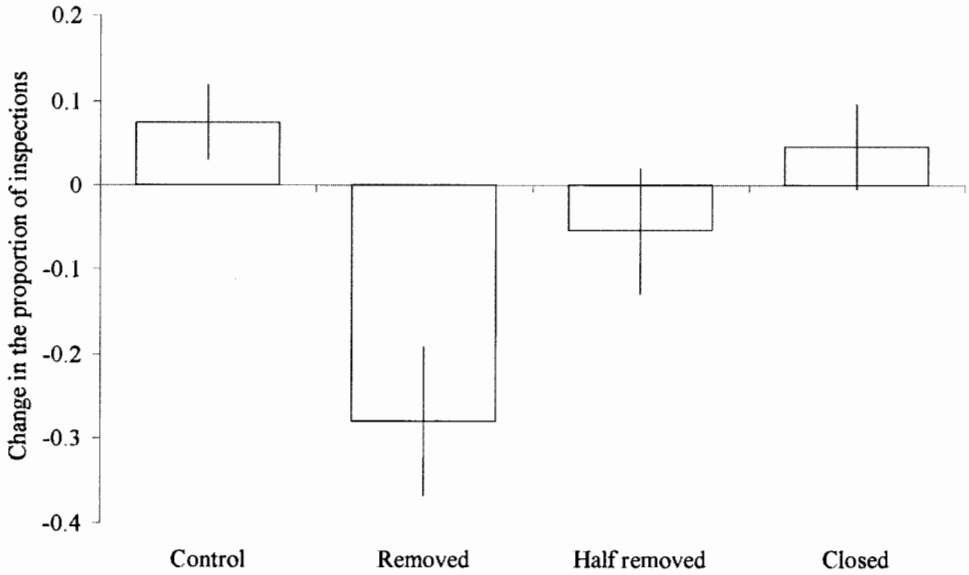




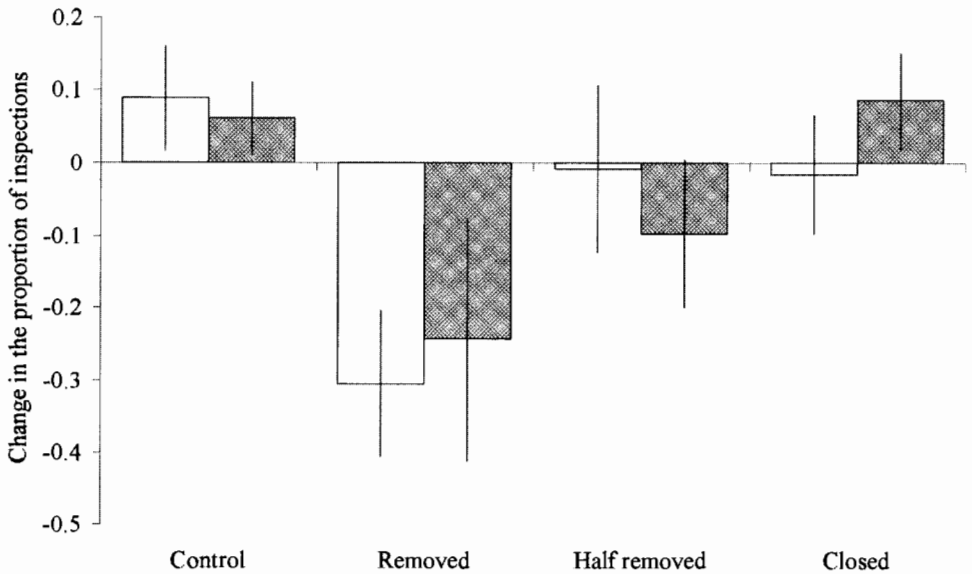
**Fig 4:** The mean (+1 SE) probability of acceptance by honeybee workers, *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] with respect to the number of previous acceptances of a lavender (*Lavandula stoechas* L) inflorescence. The number of previous visits had no effect on the probability of accepting inflorescences.

The decrease in the number of inspections for each treatment tended to be greater (though not significantly) for Treatments 2 and 3, where bracts were removed ( $F_{3,116} = 1.04$ ,  $P = 0.378$ ). There was, however, a significant difference between treatments in the change in the number of ignores ( $KW_3 = 9.373$ ,  $P = 0.025$ ): only Treatment 2 increased. The change in the proportion of the number of inspections to ignores was strongly significant ( $KW_3 = 14.07$ ,  $P = 0.003$ ): inflorescences where bracts were removed were ignored proportionally more (as found in experiment one), but there was no effect of closing the bracts (**Fig 5**). However, not all inflorescences had four bracts of equal size, and many that had four bracts had a large pair and smaller pair. Such asymmetrical bracts would be unable to conceal a spider, even when closed, so bees may not avoid them. Inflorescences that had four large bracts were separated from the others in analysis, but there was still no significant interaction with treatment in the change in the number of inspections ( $F_{3,116} = 1.203$ ,  $P = 0.312$ ), and no difference for the closed treatment alone in the change in proportion of inspections ( $KW_1 = 1.192$ ,  $P = 0.275$ ): if anything, bees were more likely to inspect inflorescences with four equal sized bracts when they were closed (**Fig 6**).

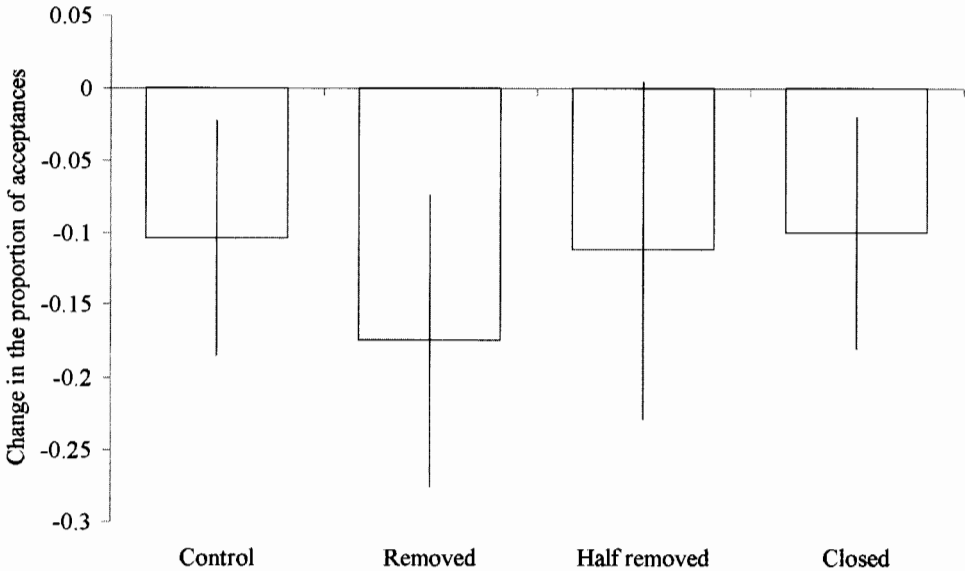
There was no difference between groups in the change in the number of acceptances ( $KW_3 = 1.733$ ,  $P = 0.621$ ) or rejections ( $KW_3 = 2.273$ ,  $P = 0.518$ ). There was no difference in the change in the probability of acceptance between groups ( $KW_3 = 0.815$ ,  $P = 0.846$ ); all inflorescences were rejected more frequently after the treatment (**Fig 7**).



**Fig 5:** The mean ( $\pm 1$  SE) change in the proportion of inspections by honeybee workers, *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] for the four treatment groups of experiment two. Lavender (*Lavendula stoechas* L) inflorescences whose bracts were removed were inspected less, but closure of the bracts had no effect.



**Fig 6:** The mean ( $\pm 1$  SE) change in the proportion of inspections by honeybee workers, *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] for the four treatment groups, separating lavender (*Lavendula stoechas* L) inflorescences with four large bracts (hatched bars) from the others (open bars). The size of the bracts had no effect.



**Fig 7:** The mean ( $\pm 1$  SE) change in the probability of acceptance by honeybee workers, *Apis mellifera* [Hymenoptera: Apidae] for the four treatment groups in experiment two. All lavender (*Lavendula stoechas* L.) inflorescences were rejected more but there were no differences between groups.

#### 4 Discussion

The results suggest that, as expected from other studies [DUFFIELD et al 1993, HERRERA 1997, HIGGINSON & BARNARD 2004, HIGGINSON et al 2006], the removal of bracts did have an effect on visitation rate. The present study shows that the effect on visitation rate to bushes or half-bushes observed previously [HERRERA 1997] is also observed when bracts are removed from inflorescences randomly within bushes. That is, the effect on the attractiveness of inflorescences acts even at short distances between inflorescences on the same plant. One possibility that could have affected the results was that damaged inflorescences smell differently to others, and if the damage released some repellent chemical this could have influenced the bees' behaviour in the observed direction. This possibility should be tested in future work.

The use of a three decision classification (*accept*, *reject*, *ignore*) enabled the novel finding that this decrease was the same for both the number of acceptances and the number of rejections, suggesting that the removal of bracts means that the bee will be less likely to *inspect* the inflorescence. This finding ties in well with earlier work [HIGGINSON et al 2006] suggesting that display size does not affect whether the bees accept after inspecting, but does strongly influence which inflorescences bees inspect, either because bees avoid small inflorescences or detect them less frequently. The bracts are an advertisement, but do not necessarily provide an honest indication of nectar content [HIGGINSON et al 2006], so, when closer, the bee uses the morphological characters that are good predictors of nectar production: age and number of flowers, or some correlate thereof (such as colour). This hypothesis is supported by the fact that the size of inflorescences before manipulation was correlated with the number of inspections, but not the number of acceptances.

Work on the visual spatial perception of bees has shown that their colour vision resolution is poor [GIURFA, VOROBYEV, KEVAN et al 1996]: Bees have only been shown to be capable of detecting an 83mm circle from a distance of 120mm [LEHRER 1997]. Since inflorescences are densely clustered in this study system bees are rarely further than a few centimetres from several inflorescences, so they are likely to be capable of judging the size of inflorescences. However, since the judgement of age and number of flowers probably requires comparison of the amount of flower colour to bract colour, bees probably need to be closer (*inspect*) to the inflorescence in order to make a final decision.

Removal of the bracts does not necessarily make the inflorescence less attractive, but the removal of the bracts could have caused the inflorescence merely to be less conspicuous to searching bees so they were less likely to detect it. An alternative, but not mutually exclusive, explanation is that the bees actively ignored inflorescences with no terminal bracts. A lack of bracts is only usually seen in immature or very old inflorescences, neither of which produces much nectar [HIGGINSON et al 2006]. Hence bees could learn by negative association that a lack of bracts indicates low reward and therefore ignore those inflorescences. It is interesting to note that the visitation rate to inflorescences with closed bracts did not fall as much as that to inflorescences with half their bracts removed, despite the fact that the reduction in visible bract area would be similar. This suggests that it is not only display size that affects inspection rate, and other factors affect the attractiveness of inflorescences, which may include colour and flower scent.

The finding that bees did not avoid visiting inflorescences that had recently been visited by a honeybee suggests that scent marking, if it occurs on *Lavandula*, does not significantly affect foraging decisions. Scent marking may be inefficient on an inflorescence bearing plant that has many bees foraging on it simultaneously, because not all flowers on a particular inflorescence are probed by bees, so a scent mark may not provide an accurate cue to the total nectar content of the inflorescence.

The results suggest that honeybees do not avoid inflorescences that have closed bracts, despite the fact that closed bracts are normally an indication that a crab spider is present. The earlier finding that the probability of accepting an inflorescence is lower if it contains a spider [READER et al 2006] is thus unlikely to be due to a closed bract cue. Bees are unlikely to have the chance to learn the association between closed bracts and the presence of a spider, because spiders frequently move between inflorescences and leave behind closed bracts. Therefore a significant proportion of inflorescences with closed bracts would not actually contain a spider, so it would be non-adaptive to avoid them all. It is likely that bees never acquire the association, since every acceptance of an inflorescence with closed bracts that does not contain a spider would weaken the association. However, in Experiment I, bees appeared to avoid closed bracts. The difference between Experiments I and II was that, in Experiment I, bracts had been closed by spiders, rather than by glue as in Experiment II. Bees may thus use some more direct mechanism for avoiding spiders, perhaps the detection of silk, and this is currently the subject of further work.

## 5 References

- ЧИТКА Л [2001]: Camouflage of predatory crab spiders on flowers and the colour perception of bees (Aranida: Thomisidae/Hymenoptera: Apidae). – *Entomol Gener* **25**: 181–187; Stuttgart/Deutschland.

- DUFFIELD G E, GIBSON R C, GILHOOLY P M, HESSE A J, INKLEY C R, GILBERT F S & BARNARD C J [1993]: Choice of flowers by foraging honey bees (*Apis mellifera*): possible morphological cues. – *Ecol Entomol* **18**: 191–197; Oxford/Britain.
- DUKAS R & MORSE D H [2003]: Crab spiders affect flower visitation by bees. – *Oikos* **101**: 157–163; København/Danmark.
- DUKAS R [2001a]: Effects of perceived danger on flower choice by bees. – *Ecology Letters* **4**: 327–333; Paris/France.
- DUKAS R [2001b]: Effects of predation risk on pollinators and plants. – In: CHITTKA L & THOMSON J D: *Cognitive ecology of pollination*. – Cambridge University Press, Cambridge/Britain.
- DYER A G & CHITTKA L [2004]: Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. – *J Comp Physiol A* **190**: 759–763; Berlin/Deutschland.
- GIURFA M & NUNEZ J A [1992]: Honeybees mark with scent and reject recently visited flowers. – *Oecologia* **89**: 113–117; Berlin/Deutschland.
- GIURFA A M & VOROBYEV M [1997]: The detection and recognition of colour stimuli by honeybees: performance and mechanisms. – *Isr J Plant Sci* **45**: 129–140; Jerusalem/Israel.
- GIURFA M, VOROBYEV M, KEVAN P & MENZEL R [1996]: Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. – *J Comp Physiol A* **178**: 699–709; Berlin/Deutschland.
- GONZALEZ A, ROWE C L, WEEKS P J, WHITTLE D, GILBERT F S & BARNARD C J [1995]: Flower choice by honey bees (*Apis mellifera* L.): sex-phase of flowers and preferences among nectar and pollen foragers. – *Oecologia* **101**: 258–264; Berlin/Deutschland.
- GOULSON D, CHAPMAN J W & HUGHES W O H [2001]: Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. – *J Insect Behav* **14**: 669–678; Berlin/Deutschland.
- HEILING A M, HERBERSTEIN M E & CHITTKA L [2003]: Crab-spiders manipulate flower signals. – *Nature* **421**: 334; London/Britain.
- HERRERA J [1997]: The role of colored accessory bracts in the reproductive biology of *Lavandula stoechas*. – *Ecology* **78**: 494–504; London/Britain.
- HIGGINSON A D & BARNARD C J [2004]: Accumulating wing damage affects foraging decisions in honeybees *Apis mellifera* L. – *Ecol Entomol* **29**: 52–59; Oxford/Britain.
- HIGGINSON A D [2005]: Effects of wing damage on the behaviour of the honeybee *Apis mellifera*. – PhD dissertation, University of Nottingham, Nottingham/UK.
- HIGGINSON A D, GILBERT F S & BARNARD C J [2006]: Morphological correlates of nectar secretion rate used by honeybees. – *Ecol Entomol* **31**: 269–276; Oxford/Britain.
- LEHRER M [1997]: Honeybees' visual spatial orientation at the feeding site. – In: LEHRER M: *Orientation and communication in arthropods*: 115–144. – Birkhäuser-Verlag; Basel/Schweiz.
- LIMA S L [1998]: Nonlethal effects in the ecology of predator-prey interactions. – *Bioscience* **48**: 25–34; Washington/USA.
- MORSE D H [1986a]: Inflorescence choice and time allocation by insects foraging on milkweed *Asclepias syriaca*. – *Oikos* **46**: 229–236; København/Danmark.
- MORSE D H [1986b]: Predatory risk to insects foraging at flowers. – *Oikos* **46**: 223–228; København/Danmark.
- PYKE G H [1979]: Optimal foraging in bumble bees *Bombus appositus*: Rules of movement between flowers within inflorescences. – *Anim Behav* **27**: 1167–1181; Palo Alto/USA.
- READER T, HIGGINSON A D, GILBERT F S, BARNARD C J & The Behavioural Ecology Field Course [2006]: The effects of predation risk from crab spiders on bee foraging behavior. – *Behav Ecol*, doi:10.1093/beheco/arl027; Oxford/Britain.
- SCHMALHOFER V R [2001]: Tritrophic interactions in a pollination system: impacts of species composition and size of flower patches on the hunting success of a flower-dwelling spider. – *Oecologia* **129**: 292–303; Berlin/Deutschland.
- SCHMID-HEMPEL P [1991]: The ergonomics of worker behaviour in social hymenoptera. – *Adv Stud Behav* **20**: 87–134; London/Britain.
- SPAETHE J, TAUTZ J & CHITTKA L [2001]: Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. – *Proc Nat Acad Sci USA* **98**: 3898–3903; Washington, USA.

WILLIAMS C S [1998] The identity of the previous visitor influences flower rejection by nectar-collecting bees. – *Anim Behav* **56**: 673–681; Palo Alto/USA.

**Acknowledgements – Danksagung:** We thank Bob Liddle and Simon Clifford for transport and subsistence and Armin Pircher and Bernd Bödeker at the Quinta de São Pedro field centre for support and entertainment in the field. Lars Chittka, and two anonymous referees provided advice on an earlier version of this manuscript. This work was funded by a BBSRC Committee Studentship to A D H.

**Authors' address – Anschrift der Verfasser:** Dr A D Higginson, Dr F S Gilbert, Dr T Reader, Prof C J Barnard, Animal Behaviour and Ecology Research Group, School of Biology, University of Nottingham, Nottingham, NG7 2RD/Britain.

Email: adhigginson@gmail.com

---

PRETE F R (Ed): **Complex Worlds from Simpler Nervous Systems.** – [464 pages, 165 illus, 16 pp color insert, 7 x 9 inch, paperback]. – MIT Press, Cambridge, USA, 2004; ISBN: 0-262-66174-8; Pr: \$ 40.00/£ 25.95. [EGR-Nr 3.026]

Unravelling the internal world of 'simple' creatures, whose neurons number several orders magnitude fewer than our own, cannot fail to tempt those looking for a suitable starting block for understanding how neural processing brings about complex behavioural phenomena. What this book highlights, however, is that evolution has had millenia to shape seemingly straightforward nervous machinery into complex, multi-level systems. The complex behavioural algorithms, abstraction and decision-making processes, planning behaviours, and integration of unique experiences presented here are a far cry from the traditional but clearly outdated view of invertebrates as simple reflex machines. Studying creatures so far removed from ourselves as a jumping spider, or a crayfish, or a butterfly, hence offers the dual attractions of providing both the opportunity to directly relate behaviour to underlying nervous systems, and an intriguing glimpse into the astounding creativity of evolution.

'Complex worlds from simpler nervous systems' approaches this task through a range of compelling stories of which each aims to explain aspects of how signal processing creates the internal world, or *Umwelt*, of a 'simpler' organism. In doing so, the authors present a tantalising range of material which touches upon refreshingly few standard model systems, instead exploring the behaviour of a diverse range of small creatures which have traditionally achieved rather less of the limelight.

The book's title is somewhat misleading, in that most chapters focus on sensory systems rather than nervous systems as a whole, and furthermore, on visual sensory systems rather than other modalities. This is reflected in the titles of the book's three sections: *Creating Visual Worlds: Abstract Representations and Algorithms*, introduced by FR PRETE; *Enhancing the Visual Basics: Using Color and Polarization*, introduced by CRONIN; and *Out of Sight: Creating Extravisual Worlds*, introduced by VAN STAADEN. However, several of these contributions provide excellent reviews of their subject area, and would appeal to those with a wider interest than vision alone. Strong chapters on acoustic processing in grasshoppers (VAN STAADEN et al), mechanoreception in cockroaches and crickets (COMER & LEUNG), and honeybee cognition (ZHANG & SRINIVASAN) also go some way towards redressing this imbalance. Of the rest, a particular highlight is a chapter by HARLAND and JACKSON, which successfully immerses the reader in the perceptual world of the jumping spider genus *Portia*.

Together, these stories will provide an informative read for anyone interested in the boundaries and possibilities of neural systems of 'simple' and 'advanced' creatures alike. As PRETE points out in his introduction, in these little animals, we can see the beginnings of the complex intellectual processes that define us as human.

ELLOUISE LEADBEATER [Queen Mary University of London]