

## Seeing is believing: information content and behavioural response to visual and chemical cues

Francisco G. González and Miguel A. Rodríguez-Gironés

*Proc. R. Soc. B* 2013 **280**, 20130886, published 22 May 2013

---

### Supplementary data

["Data Supplement"](#)

<http://rspb.royalsocietypublishing.org/content/suppl/2013/05/17/rspb.2013.0886.DC1.html>

### References

[This article cites 39 articles, 6 of which can be accessed free](#)

<http://rspb.royalsocietypublishing.org/content/280/1763/20130886.full.html#ref-list-1>

### Subject collections

Articles on similar topics can be found in the following collections

[behaviour](#) (959 articles)

[cognition](#) (205 articles)

[ecology](#) (1337 articles)

### Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)



CrossMark  
click for updates

## Research

**Cite this article:** González FG, Rodríguez-Gironés MA. 2013 Seeing is believing: information content and behavioural response to visual and chemical cues. *Proc R Soc B* 280: 20130886. <http://dx.doi.org/10.1098/rspb.2013.0886>

Received: 8 April 2013

Accepted: 30 April 2013

### Subject Areas:

behaviour, cognition, ecology

### Keywords:

chemical perception, predator–prey interactions, predation risk, predator detection, terrestrial insects, visual perception

### Author for correspondence:

Francisco G. González

e-mail: [franciscog.gonzalez@gmail.com](mailto:franciscog.gonzalez@gmail.com)

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2013.0886> or via <http://rspb.royalsocietypublishing.org>.

# Seeing is believing: information content and behavioural response to visual and chemical cues

Francisco G. González and Miguel A. Rodríguez-Gironés

Estación Experimental de Zonas Áridas, EEZA-CSIC, Ctra. de Sacramento S/N, La Cañada de San Urbano, 04120 Almería, Spain

Predator avoidance and foraging often pose conflicting demands. Animals can decrease mortality risk searching for predators, but searching decreases foraging time and hence intake. We used this principle to investigate how prey should use information to detect, assess and respond to predation risk from an optimal foraging perspective. A mathematical model showed that solitary bees should increase flower examination time in response to predator cues and that the rate of false alarms should be negatively correlated with the relative value of the flower explored. The predatory ant, *Oecophylla smaragdina*, and the harmless ant, *Polyrhachis dives*, differ in the profile of volatiles they emit and in their visual appearance. As predicted, the solitary bee *Nomia strigata* spent more time examining virgin flowers in presence of predator cues than in their absence. Furthermore, the proportion of flowers rejected decreased from morning to noon, as the relative value of virgin flowers increased. In addition, bees responded differently to visual and chemical cues. While chemical cues induced bees to search around flowers, bees detecting visual cues hovered in front of them. These strategies may allow prey to identify the nature of visual cues and to locate the source of chemical cues.

## 1. Introduction

Foraging animals can reduce mortality using sensory information to evaluate predation risk and reducing exposure to predators [1]. Predator avoidance has important ecological and evolutionary consequences, as it can affect the pattern of interactions within communities [2] and determine the strength of trophic cascades through indirect behavioural effects [3]. Nonetheless, research on this topic presents important biases and some aspects of how animals use sensory information to assess predation risk have been relatively neglected. Thus, although information about the presence of predators is received through several sensory channels, mostly in form of visual and chemical cues [4], most studies of anti-predator decision making consider a single sensory modality [5]. Besides, research on how animals use different sensory modalities to assess predation risk has focused mainly on the threat-sensitivity (graded response in relation to the magnitude of risk) [6] and sensory-compensation (compensation for poor information in one sensory modality by increasing reliance on another sense) [7] hypotheses—which revolve around the ideas that redundancy increases the reliability of cues, and that the efficiency of cues is habitat-specific.

If it is true that information loss during transfer depends on the sensory channel combination, a more important difference is that visual and chemical cues convey different information [8]. Yet, little effort has been devoted to study the information content of predator-related cues and the extent to which different cues trigger different behavioural responses—rather than responses of different intensity. Although visual cues have higher resolution in time and space [9,10], for many taxa they are also more ambiguous than chemical cues [11]. In particular, animals with poor visual resolution may find it difficult to discriminate between predatory and harmless species on the basis of visual cues alone. We should, therefore, expect prey to respond differently, and not just at different intensities, to the presence of

visual and chemical cues: if the nature of the cue is ambiguous, we would expect prey to seek confirmation of potential threats; whereas if the ambiguity resides in the spatiotemporal origin of the cue, prey should try to locate the predator.

Solitary bees constitute an ideal system to study the mechanisms of predator avoidance. Bees use chemical and visual information during foraging [12]. Particularly in solitary bees, there is a very tight link between foraging efficiency, predator avoidance and fitness [13]. As a result, bees have evolved predator-avoidance strategies [3,14–18] and respond to the trade-off between minimizing predation risk and maximizing foraging efficiency [19]. Furthermore, it is known that chemical information plays an important role in risk assessment: the response of bees to ambushing crab spiders changes when chemical cues are removed [20], and social bees release alarm pheromones to mark flowers where a predator is hidden [14,21]. Finally, it is easy to study bees in their natural habitat without interfering with their foraging activity. Studying predator avoidance in the field—rather than in the laboratory—is important because animals can perceive the laboratory environment as very dangerous, a perception that can affect the outcome of experiments [7,22].

While empirical studies on the use of different sensory modalities to assess predation risk have focused on the issues of additivity and sensory compensation, theoretical investigations of anti-predator behaviour normally look at the relationship between predation risk and optimal behaviour [23]. Models typically assume that prey have perfect information of predation risk and ignore the process of risk assessment [24]. In this paper we concentrate on how prey use predator-related visual and chemical cues to detect, assess and respond to predation risk. To integrate our work into an optimal foraging perspective, we first developed a mathematical model to determine how environmental parameters affect the time that bees should spend examining flowers prior to deciding whether to land on them. We then examined in the field the behaviour of female solitary bees, *Nomia strigata*, visiting flowers with and without visual and chemical cues from predatory, *Oecophylla smaragdina*, and harmless, *Polyrhachis dives*, ants. We had previously confirmed, using gas chromatography-mass spectrometry (GC-MS), that the two ant species differed in the profile of cuticular volatiles they emitted. If bees were able to discriminate between the two ant species, they should treat *P. dives* flowers as safe flowers. Otherwise, they should treat them as dangerous flowers. The model predicted that bees should spend more time examining flowers that they perceive as riskier and that the probability of false alarms should decrease from early morning to noon as resources become depleted. As for the nature of the response, chemical cues indicate that a predator is, or has been, near the flower [8]. Chemical cues should, therefore, induce bees to search for predators. Visual cues, on the other hand, indicate that the flower is occupied by another individual. In response to visual cues, bees should attempt to determine whether the flower occupant is dangerous or harmless.

## 2. Material and methods

### (a) Model description

In this section, we offer a verbal description of the model. A detailed mathematical treatment is provided as electronic

supplementary material, appendix SI. The aim of the model is to calculate the predator-avoidance strategy that maximizes the expected fitness of female solitary bees. We first develop a basic model to study the optimal relationship between examination time and environmental parameters, ignoring the effect of distinct predator cues. We then extend the basic model in two directions: introducing predator cues and variability in the reward offered by different flowers.

The basic model assumes that, upon approaching a flower, bees can spend a certain time examining it prior to deciding whether to land or move on and search for a new flower. Flower examination decreases the uncertainty concerning the presence or absence of a predator at the flower, and given enough time a bee could be certain that the flower does, or does not, harbour a predator. However, a bee that spends a long time examining each flower will visit few flowers per day—there is a trade-off between increasing intake rate and decreasing predation risk—so the optimal strategy will normally consist of collecting only partial information. We model information acquisition as a Bayesian-like process. Upon approaching a flower, bees have a prior expectation that the flower harbours a predator. This prior expectation depends on the abundance of predators in the environment. Information is updated during flower examination, leading to a posterior expectation that the examined flower harbours a predator. Because examination is a stochastic process, the posterior expectation is not fully determined by the presence or absence of a predator at the flower. Rather, it is a random variable, and its mean and variance depend on whether the flower harbours a predator, and on the amount of time invested in examining the flower. Longer times lead to more accurate expectations. Finally, the predator-avoidance strategy of bees, in this cue-free basic model, is defined by two parameters: flower examination time and a rejection criterion—essentially, a value of the perceived level of threat above which bees abandon flowers without landing.

Ignoring other sources of mortality and resource limitation, the expected fitness of a female solitary bee will be the number of eggs she can provision before dying [13]. We assume that bees have a fixed lifespan, so that the number of eggs they can lay, if they manage to avoid predation, is determined by the time required to provision eggs. The two components of the predator-avoidance strategy affects this time. Lower rejection criteria lead to a higher number of false alarms (rejected safe flowers), and hence higher provisioning times because bees must visit more flowers to provision an egg. The effect of examination time on provisioning, however, is more subtle: longer examination times tend to increase provisioning time, because bees spend more time per flower, but they can decrease the number of false alarms, shortening provisioning times. At the same time, however, increasing the examination time and lowering the rejection criterion increase the probability of surviving to rear an offspring. Expected fitness is an increasing function of this probability of surviving and a decreasing function of the time required to provision an egg [25]. The model is restricted to solitary bees because the relationship between foraging efficiency, survival and fitness differs between social and solitary bees [13,26]. We consider two extensions of the basic model.

First, we study how bees should respond to the detection of cues (see the electronic supplementary material, appendix SIb). For simplicity, we assume that cue detection is an immediate, all-or-none process. Furthermore, we consider a single cue type, ignoring any difference in the information that visual and chemical cues may convey. An explicit treatment of these differences would require the development of a cognitive model and is beyond the scope of this paper. Bees may detect predator cues at safe and dangerous flowers, although the probability of detecting them is greater at dangerous flowers. Thus, bees may detect visual cues at safe flowers if a harmless insect walks on the petals, and they can detect chemical cues if a predator is, or

has recently been, in the neighbourhood of the flower they approach. Likewise, bees may fail to detect cues when approaching dangerous flowers. The predator may be hidden in the underside of the flower, and the wind may blow volatiles away from the approaching bee. Within this framework, we derive the optimal response of bees when the probability of detecting a cue is  $0.5 + \rho$  if the flower harbours a predator and  $0.5 - \rho$  otherwise. Note that the parameter  $\rho$  is tightly linked to the reliability of the cue: when  $\rho = 0$ , cues convey no information. On the other hand, when  $\rho = 0.5$ , cues become maximally informative.

Finally, we extend the basic model to study the optimal strategy of bees when they encounter rich and poor flowers, differing in the amount of resources they offer—in the absence of predator cues. We assume that bees can discriminate between rich and poor flowers once they are sufficiently close to them, although bees cannot discriminate between rich and poor flowers at a distance [27], so that they encounter flowers at random (that is: bees cannot specialize in visiting only rich flowers). In this situation, the foraging strategy of bees consists of four parameters: two examination times and two rejection criteria (one pair of parameters for each flower type, rich and poor). As in the previous case, these parameters determine the probabilities of landing at safe and dangerous flowers, and from these probabilities, we can calculate average time required to provision an egg and the probability of surviving through egg provisioning. From these quantities, we can easily compute expected fitness (see the electronic supplementary material, appendix S1c).

The biology of our model species, *N. strigata*, is not known with sufficient detail to estimate the parameters involved in the model. For this reason, we can only use our model to make qualitative predictions (see the electronic supplementary material, appendix S1d). Although we cannot experimentally measure the rejection threshold of bees, we can measure examination times and proportion of false alarms, and compare them with model predictions. Thus, we can evaluate how examination times and proportion of false alarms should change with the overall predation risk, with the conspicuousness of predators, or the number of flowers that bees must exploit in order to provision an egg.

## (b) Study site and species

We tested the model comparing the behaviour of solitary bees, *N. strigata*, exploiting nectarless *Melastoma malabathricum* flowers with and without cues associated to the presence of two ant species: the predatory weaver ant, *O. smaragdina* and the harmless *P. dives*. We run two independent experiments to study the effect of chemical and visual cues. We selected this system because small bees avoid rambutan trees (*Nephelium lappaceum*) with *O. smaragdina* but not with *P. dives* ants [28] and, in particular, *N. strigata* bees exhibit strong anti-predator behaviour when exploiting flowers at *M. malabathricum* plants harbouring *O. smaragdina* nests [3]. We carried out all experimental work at MacRitchie Reservoir in the Central Catchment Nature Reserve, Singapore, from early June to late July 2010 and within a geographical range of 100 m. Because *N. strigata* seldom forages at plants with weaver ant nests [3], all observations were conducted at ant-free plants on sunny, windless days during the peak activity period of *N. strigata* (8:30–12:00). *Nomia strigata* visit rate at ant-free plants was on average 10.2 bees  $\text{h}^{-1}$  per flower. Weaver ants were the only predators that we observed attacking *N. strigata* bees in our study site.

## (c) Effect of chemical cues

GC-MS analysis revealed that the two ant species differed in the volatile mixtures they emitted (see the electronic supplementary material, appendix S1I and figure S1), offering bees the possibility to use chemical information to discriminate between them and detect predatory ants.

We bagged flowers before anthesis to prevent pollen removal by foraging bees and scent contamination by bees or patrolling ants [29]. Using forceps, we placed a living ant (*O. smaragdina* or *P. dives*, depending on the treatment) inside each bag. The ant was free to patrol the flower surface for at least 1 h. When it was time to use a flower for the observations, we removed both bag and ant, cut off the floral stem, fastened it with clips to an ant-free shrub and videotaped the arrival of the first *N. strigata* bee to the flower. Flowers in the control treatment were subject to the same manipulation, except that no ant was introduced in the bag. Each treatment was replicated 50 times. Flowers and ants were used only once (i.e. we recorded a single bee visit per flower), and flowers were allocated to treatment sequentially (one flower per treatment), to prevent correlations between time and treatment. Although bees were not marked, to minimize the probability of using the same bee several times we conducted observations at spatially separate plants. We observed one flower at a time.

## (d) Effect of visual cues

We used dead ants as visual cues. Ants (*O. smaragdina* or *P. dives*, depending on the treatment) were killed by freezing and kept for two days in a well-ventilated room before the observations. GC-MS analysis confirmed that dead ants did not differ in the cuticular volatiles they emitted (see the electronic supplementary material, appendix S1I and figure S2), so that bees could not use chemical cues to discriminate between them. On the other hand, humans can easily discriminate between the two species by sight. Body size and colour are the most conspicuous differences: *O. smaragdina* ants (mean  $\pm$  s.d.:  $9.50 \pm 0.32$  mm,  $n = 20$ ) were longer than *P. dives* ants ( $5.60 \pm 0.26$  mm,  $n = 20$ ), and this difference was a reliable indicator of ant species ( $t$ -test:  $t_{38} = 41.65$ ,  $p < 0.0001$ ). *Oecophylla smaragdina* ants were also more brownish than *P. dives* ants (see the electronic supplementary material, figure S3). In principle, then, bees could use visual cues, such as body size, to discriminate between the two ant species.

We bagged ant-free flowers as explained above. When it was time to use a flower, we removed its bag, cut off the floral stem, fastened it with clips to an ant-free shrub, glued a dead ant with a drop of Loctite Super Glue to the middle of a haphazardly selected petal of the flower and recorded the arrival of the first *N. strigata* bee to the flower. Control flowers had a drop of glue but no dead ant. Each treatment was replicated 50 times.

## (e) Video analysis

We recorded the arrival of the first bee with a Sony DCR-SR4 Handycam camcorder. Using Picture Motion Browser, we extracted the following information from each video: examination time, and presence or absence of hovering, exploration and rejection. Examination time was quantified as the time elapsed since bees first approached within approximately 5 cm of flowers until they either landed on them or left them to search for another flower. We selected 5 cm as a standard distance from flowers because it was the approximate length at which bees halted their approach flight. Nevertheless, because the initial approach of bees to flowers was very fast, changing this distance would lead to very minor changes in examination time. We adopted the following operational definitions of hovering, exploration and rejection. A bee hovered when she performed side-to-side scans [30] in front of ants/petals during at least one second (see the electronic supplementary material, movie S1). A bee explored a flower when she approached it within 5 cm and then performed flights (not necessarily within 5 cm of the flower) under and/or around the flower (see the electronic supplementary material, movie S2). Flower approaches ended when the bee 'landed' on the flower to exploit its pollen or 'rejected' the flower and moved on to another one. Examination time was a continuous variable, whereas

hovering, exploration and rejection were binomial variables (i.e. we scored whether bees did or did not perform these behaviours).

## (f) Statistical analyses

We used generalized linear models (GLM) to study the effect of time of day, cue type (visual versus chemical), ant species (*O. smaragdina*, *P. dives* or no ant—control) and the interaction between cue type and ant species on the following response variables: examination time, probability of rejecting flowers, probability of hovering and probability of exploring flowers. For the examination times, the GLM had gamma distribution and inverse link function, whereas for the probabilities they had binomial distribution and logit link function. Unless otherwise specified, analyses were performed using R v. 2.15.0 [31]. We applied type II log likelihood ratio (LR) tests—function ANOVA (model, type = 'II', test.statistic = 'LR')—to calculate significance levels and used planned contrasts—function contrast (model, list 1, list 2)—to make pairwise comparisons between groups.

To compare examination times prior to the rejection or acceptance of flowers with dead *O. smaragdina* ants, we performed a GLM on log-transformed data, using bee decision (rejection versus acceptance) as fixed factor and time of day as covariate.

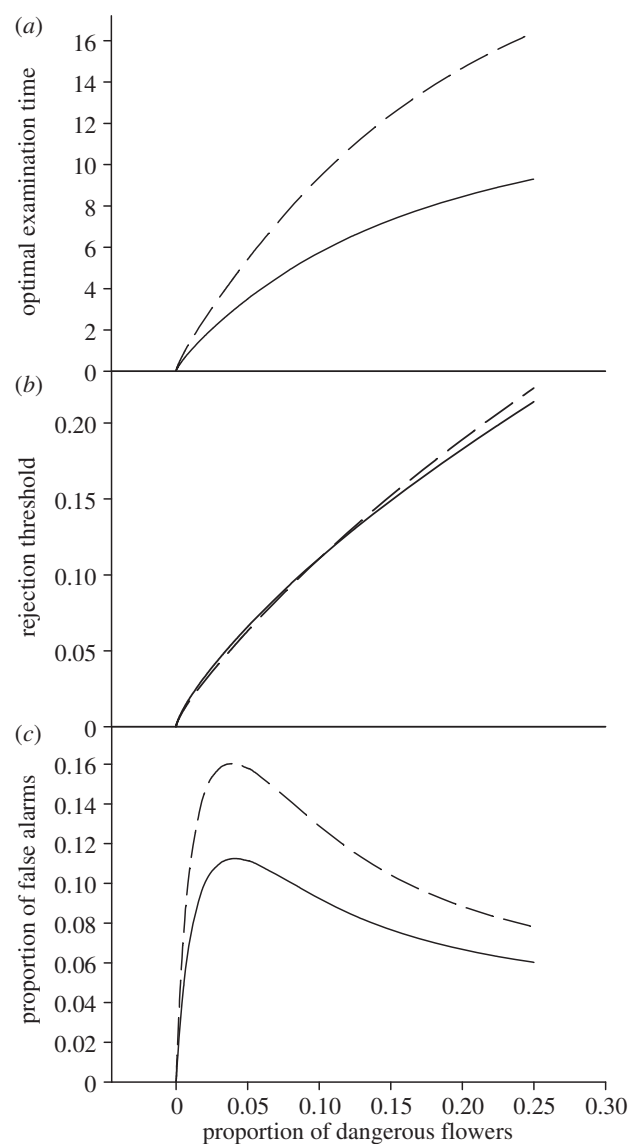
In four out of six experimental groups, all bees eventually landed on the flower they approached, preventing the algorithm implemented by R to converge. For this particular test, we, therefore, calculated model likelihood as explained by [32], set the probabilities of rejection equal to 0 in the four groups where all flowers were accepted and estimated the remaining parameters using the Solver command of Microsoft EXCEL 2010. As in previous cases, we used LR tests to calculate significance levels. Data are available in the electronic supplemental material (see the electronic supplementary material, table S1).

## 3. Results

### (a) Model results

The optimal values of the examination time and rejection threshold increased with the proportion of dangerous flowers, and examination time was greater for cryptic than for conspicuous predators (figure 1*a,b*). We can, therefore, use examination time as an indicator of risk assessment: examination times should increase with the level of threat perceived by bees. The proportion of false alarms (the proportion of safe flowers that bees reject after examination) attained a maximum for intermediate values of the proportion of dangerous flowers and was also greater for cryptic than conspicuous predators (figure 1*c*).

Surprisingly, the optimal predator-avoidance response was essentially independent of the number of flowers that bees must exploit to provision an offspring, although expected fitness increased as the number of flowers that must be exploited decreased (data not shown). These results imply that bees should behave similarly in rich environments, with a high level of reward per flower, and poor environments, where many more flowers must be exploited to provision an offspring. This, however, does not mean that bees should pay no attention to the reward offered by flowers, as shown by the predictions of the model for heterogeneous environments. When, within one environment, flowers differed in the reward they offered, optimal examination times and rejection thresholds were relatively similar at both flower types. For the particular example we considered, examination times were typically 10 per cent longer at more rewarding flowers, and rejection thresholds



**Figure 1.** Effect of predator abundance and conspicuousness. Optimal examination time, in seconds (*a*), rejection threshold (*b*) and proportion of false alarms (*c*) for different abundances of conspicuous ( $k_s = 0.1$ ,  $k_d = 0.5$ ; solid line) and cryptic ( $k_s = 0.05$ ,  $k_d = 0.25$ ; dashed line) predators.

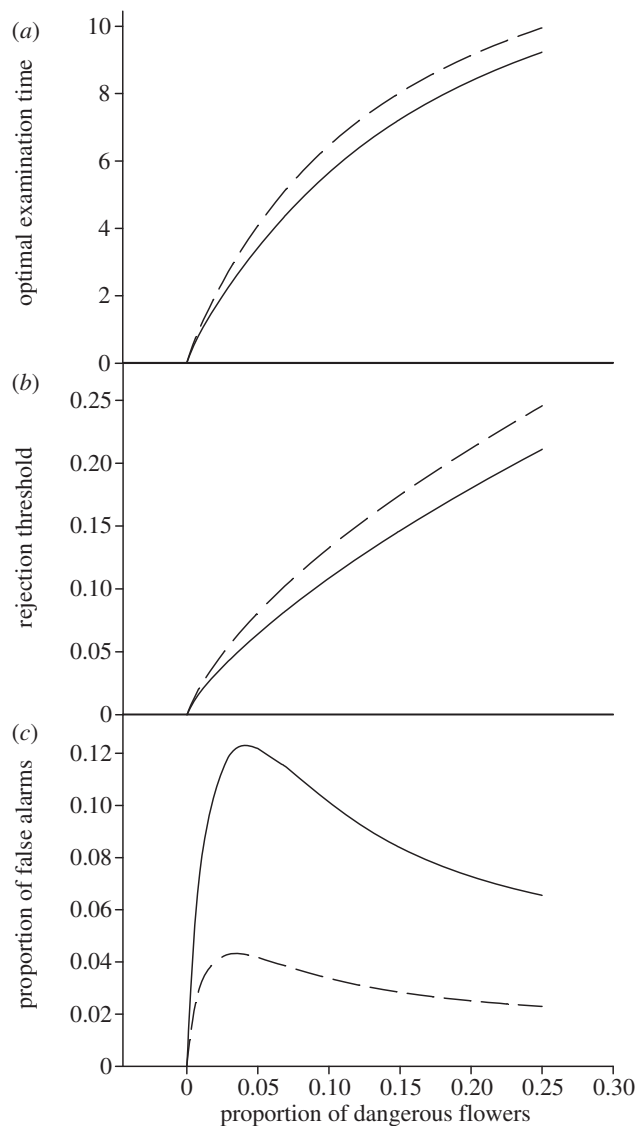
about 20 per cent greater (figure 2*a,b*). The rate of false alarms, however, was almost three times greater at poor than at rich flowers (figure 2*c*).

When the probabilities that safe and dangerous flowers were associated with predator cues were given by  $0.5 - \rho$  and  $0.5 + \rho$ , respectively, the value of  $\rho$  determined the conditional probability that a flower where cues had, or had not, been detected harboured a predator. When  $\rho$  increased, the probability that a flower was dangerous decreased if no cues were detected and increased if cues were detected. As a result, the optimal examination time increased with  $\rho$  at flowers where cues were detected and decreased at flowers where no cues were detected (figure 3).

## (b) Experimental results

### (i) Examination times

We recorded the approach of *N. strigata* bees to unvisited *M. malabathricum* flowers with a dead ant glued to a petal (visual cue) or to flowers impregnated with the smell of ants

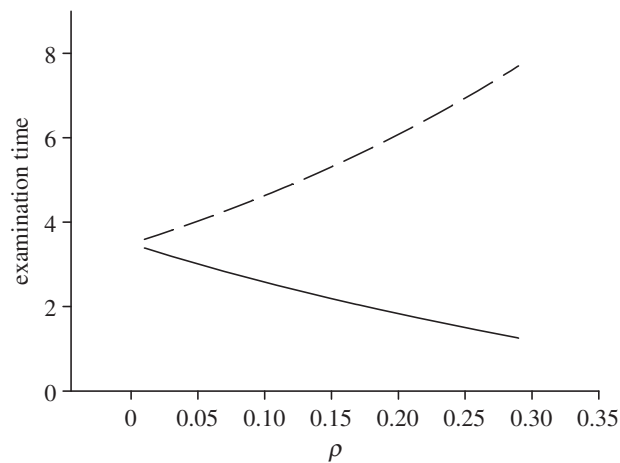


**Figure 2.** Effect of variability in reward. Optimal examination time, in seconds (a), rejection threshold (b) and proportion of false alarms (c) for poor (solid line) and rich (dashed line) flowers in a heterogeneous environment.

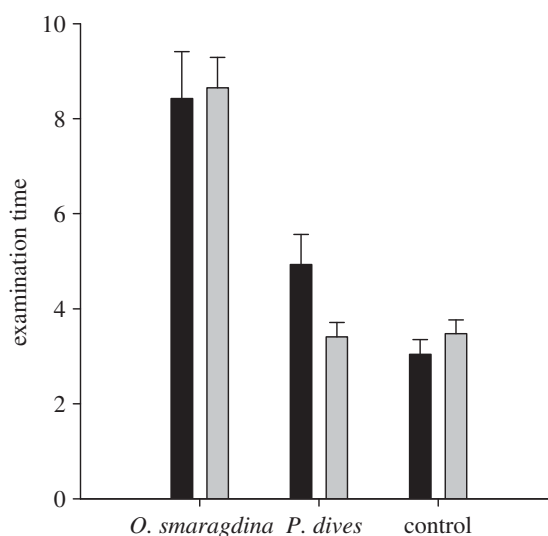
(chemical cue). Time of day had no effect on examination time (LR:  $\chi^2_1 = 0.97$ ,  $p = 0.32$ ), but there was a significant effect of the interaction between ant species and cue type on examination time (LR:  $\chi^2_2 = 7.14$ ,  $p = 0.028$ ): examination times were longer at flowers with *O. smaragdina* cues than at control flowers or flowers with *P. dives* cues, but examination times at control flowers and flowers with *P. dives* cues only differed for visual cues (figure 4). Indeed, planned contrasts showed that examination times were longer when bees approached flowers with *O. smaragdina* cues than when they approached flowers with *P. dives* cues, regardless of whether the flowers contained visual ( $t_{294} = 3.54$ ,  $p = 0.0005$ ) or chemical ( $t_{294} = 5.57$ ,  $p < 0.0001$ ) cues. Furthermore, examination times at flowers with *P. dives* visual cues were longer than at control flowers ( $t_{294} = 3.22$ ,  $p = 0.0014$ ), but there was no difference in examination times at control flowers and flowers with *P. dives* chemical cues ( $t_{294} = 0.14$ ,  $p = 0.89$ ).

### (ii) Nature of behavioural response

The probability that *Nomia* bees explored flowers was independent of time of day (LR:  $\chi^2_1 = 0.13$ ,  $p = 0.72$ ), but was



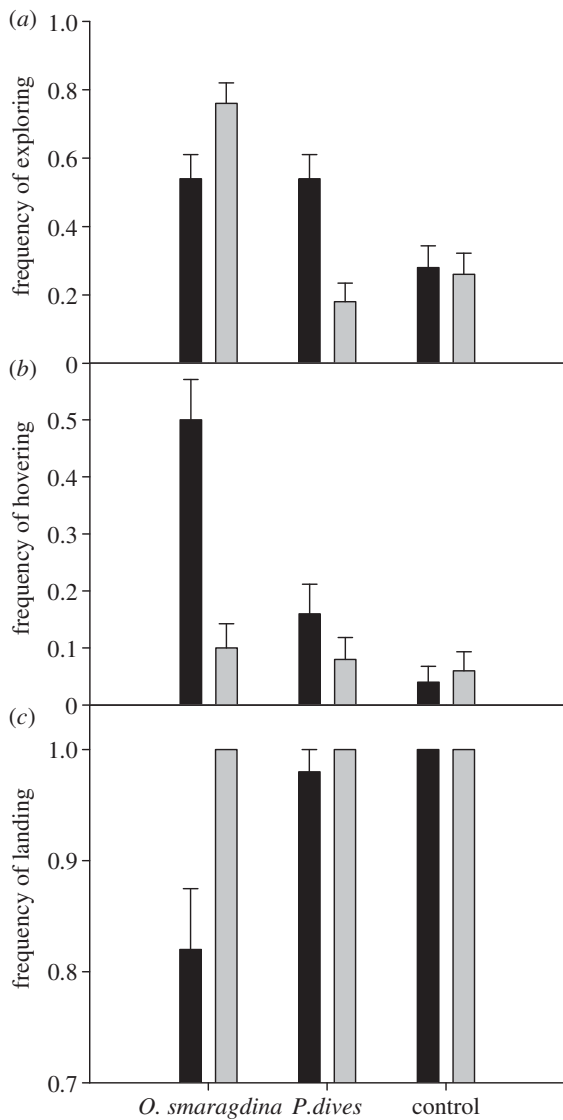
**Figure 3.** Expected effect of cue detection. Examination time at flowers where predator cues are detected (dashed line), and where no cue is detected (solid line) as a function of  $\rho$ . Parameter values as for conspicuous predators in figure 1.



**Figure 4.** Average examination times (s) of *Nomia* bees at flowers with visual (black bars) and chemical (grey bars) cues from *O. smaragdina* and *P. dives* ants and control flowers. Error bars represent 95% confidence intervals (CIs).

affected by the interaction between ant species and cue type (LR:  $\chi^2_2 = 19.00$ ,  $p < 0.0001$ ; figure 5a): the probability of exploring flowers was similar when bees encountered *O. smaragdina* or *P. dives* visual cues ( $t_{294} < 0.01$ ,  $p > 0.99$ ), but in the presence of chemical cues the probability of exploring flowers was much higher if the cues were associated with *O. smaragdina* than with *P. dives* ants ( $t_{294} = 5.39$ ,  $p < 0.0001$ ). The probability of exploring was similar for bees encountering control flowers or flowers with *P. dives* chemical cues ( $t_{294} = -0.96$ ,  $p = 0.34$ ), but bees encountering flowers with *P. dives* visual cues were more likely to explore than bees encountering control flowers ( $t_{294} = 2.61$ ,  $p = 0.01$ ). Finally, when bees encountered flowers with *O. smaragdina* cues, they were more likely to explore them if flowers had chemical than if they had visual cues ( $t_{294} = -2.28$ ,  $p = 0.02$ ).

We turn now to the hovering response. Bees were more likely to hover early in the morning than towards noon (LR:  $\chi^2_1 = 4.66$ ,  $p = 0.03$ ), and there was a significant effect of the interaction between cue type and ant species on the



**Figure 5.** Frequency of bees (a) exploring under flowers and/or performing fast movements around them, (b) hovering in front of flowers and (c) landing on flowers, with visual (black bars) and chemical (grey bars) cues from *O. Smaragdina* and *P. dives* ants and control flowers. Error bars represent 95% CIs.

probability of hovering (LR:  $\chi^2_2 = 7.45$ ,  $p = 0.02$ ; figure 5b). This interaction stems from a much greater difference in the probability of hovering between visual and chemical *O. smaragdina* cues ( $t_{294} = 4.00$ ,  $p = 0.0001$ ) than between visual and chemical *P. dives* cues ( $t_{294} = 1.21$ ,  $p = 0.2276$ ). Furthermore, bees were more likely to hover in front of a dead *O. smaragdina* than *P. dives* ant ( $t_{294} = 3.47$ ,  $p = 0.0006$ ), whereas the probability of hovering in front of a flower with chemical cues from either species was as low as for control flowers (*O. smaragdina* versus *P. dives*:  $t_{294} = 0.35$ ,  $p = 0.73$ ; *P. dives* versus control:  $t_{294} = 0.39$ ,  $p = 0.70$ ).

### (iii) False alarms

All bees approaching flowers with chemical cues eventually landed on them (figure 5c). For bees in the visual-cue treatment, all bees accepted control flowers, but one bee (out of 50: 2%) rejected a flower with a dead *P. dives* ant, and the number of bees rejecting flowers was greatest (nine out of 50: 18%) for flowers with a dead *O. smaragdina* (figure 5c).

Both cue type (LR:  $\chi^2_1 = 17.65$ ,  $p < 0.0001$ ) and ant species (LR:  $\chi^2_2 = 16.07$ ,  $p = 0.0003$ ) had statistically significant effects on the probability that bees rejected flowers, but the effect of their interaction was not significant (LR:  $\chi^2_2 < 0.01$ ,  $p > 0.99$ ). The probability that bees rejected flowers was greatest in the early morning and decreased as the morning progressed, the effect of time of day being statistically significant (LR:  $\chi^2_1 = 5.08$ ,  $p = 0.024$ ).

When flowers had a dead *O. smaragdina* ant, examination times were shorter for bees that rejected the flower ( $4.97 \pm 4.38$  s.) than for bees that eventually landed on the flower ( $9.18 \pm 7.22$  s.), a difference that was statistically significant ( $F_{1,47} = 6.76$ ,  $p = 0.012$ ).

## 4. Discussion

Rather than asking whether the response of prey species to different predator cues is additive, or depends on environmental condition, our experiments were designed to test whether the nature of the response depends on the information conveyed by the cues. As predicted, we found that bees spent more time examining flowers with cues from the predatory ant, *O. smaragdina*, than flowers without cues or with cues from a harmless ant, *P. dives*. Besides, bees engaged in different behaviours in response to visual and chemical cues: bees hovered in front of visual cues and explored flowers where they had detected a chemical cue. Presumably, these responses helped bees to determine whether visual cues corresponded to a predator and to locate the source of the chemical cues.

The model we have presented can help us understand a number of results, such as the increase in examination time in response to predator cues and the decrease in the rate of false alarms from early morning towards noon. Optimal examination time increases with the expectation, previous to examination, that the flower harbours a predator (figure 1). In the absence of cues, this expectation is simply the proportion of flowers harbouring predators in the patch or population. When cues are detected, however, the expectation that the flower harbours a predator exceeds the proportion of predator-harboring flowers, triggering an increase in optimal exploration time (figure 3). The relationship between time of day and proportion of false alarms is less intuitive. While experiments were conducted on bagged, virgin flowers with a full pollen crop, the amount of pollen available at open flowers decreased exponentially with time of day [3]. Early in the morning, when flowers open, bees forage in a homogeneous, rich environment. However, as resources become depleted, the environment becomes more and more heterogeneous, with experimental flowers offering more resources than the average flower. Because, in heterogeneous environments, the frequency of false alarms should be greater for poor than for rich flowers (figure 2), the probability of false alarms should decrease from early morning to noon, as the relative value of experimental flowers increases. It should be noted, however, that we cannot conclude from our observation that the decrease in the rate of false alarms was owing to an increase in the relative value of previously unvisited flowers. Additional work would be required to confirm this mechanistic link.

The model also predicted results from previous experiments, such as the increase in exploration time and in the

rate of false alarms, when predators are cryptic [19]. The model, however, failed to explain other results, and these failures are as informative as its successes. The model did not predict that visual and chemical cues should trigger different response types. By contrast, it predicted an increase in examination time, from morning to noon, which was not observed. These failures underline the need to develop dynamic cognitive models of predator avoidance. Thus, the model assumed that flower examination time would be pre-determined upon arrival to a flower. In practice, it makes more sense to abandon a flower as soon as a predator has been unambiguously detected. If we are to include this feature into a model, however, the model should explicitly address the dynamic nature of information acquisition. Likewise, our model did not incorporate differences in information content from different cue types, or the possibility of choosing among several behaviours in response to perceived cues. It could, therefore, not predict that visual and chemical cues would trigger different responses. A cognitive model is better suited to incorporate these nuances. A cognitive model would also allow the incorporation of features such as learning and memory, which have been shown to play a role in predator avoidance [19,30,33]. If applying a cognitive model to explain the behaviour of insects may, at first, seem odd, we should keep in mind that insects are capable of performing complex cognitive tasks, such as rule learning and categorization [34] or numerosity [35,36]. Indeed, it has been argued that brain size is more likely to be correlated with the degree of detail and precision of perception than with cognitive capacity [37].

In our experiments, bees responded differently to visual and chemical cues associated with predatory ants. Although bees increased the time they spent examining flowers prior to landing in response to both cue types (figure 4), the behaviours they engaged during flower examination depended on the type of cue perceived. Upon detection of a chemical cue, most bees engaged in exploratory behaviour, flying around and under the flower (figure 5*a*). Because chemical cues have little spatiotemporal resolution [8], detection of these cues does not necessarily imply that the flower harbours a predator—the predator may be in a nearby flower or may be already gone. It makes, therefore, sense to determine whether the predator represents a threat before abandoning the flower. Interestingly, chemical cues never led to flower rejection—bees only rejected flowers when dead ants were present. Upon detection of visual cues, however, some bees engaged in exploration and others hovered in front of the dead ant (figure 5*b*). The side-to-side scans performed during hovering probably helped bees fixate the image, contributing to the discrimination between predatory and harmless ants [30].

A key step of threat assessment is the discrimination between predatory and harmless species [38]. Certain cues can be used to identify as predators individuals belonging to different taxonomic groups. Thus, a volatile in the urine of most mammalian carnivore predators triggers predator-avoidance responses in mice [39]. When predatory and harmless species are closely related, however, discrimination between them may be more difficult. For instance, wall lizards, *Podarcis muralis*, presented with visual and chemical cues were unable to discriminate between predatory and harmless snakes when entering a refuge [40]. In this respect, we can conclude that chemical cues provide reliable information about predator

identity: *N. strigata* bees responded to chemical cues from the predatory *O. smaragdina* ant, but not from the harmless *P. dives* ants (figures 4 and 5). Given the distinctness of the bouquet of cuticular volatiles from the two species (see the electronic supplementary material, figure S1) and the highly developed scent-recognition system of bees [12], this difference was to be expected. Visual cues, however, were less specific: visual cues from harmless ants triggered a predator-avoidance response, similar in nature, although weaker, to the response triggered by predatory ant visual cues (figures 4 and 5). Once again, this result is consistent with the limited spatial resolution and image-forming capabilities of the bee's visual system [41].

Although the use of chemical information about risk by terrestrial insects has been questioned [9], our results add to the growing evidence that chemical cues play a key role in predator avoidance by terrestrial insects. For instance, it has been reported that chemical cues from *O. smaragdina* deter fruit flies from ovipositing in mangoes on which ants have patrolled [42], and that social bees mark with alarm signals flowers where they have been attacked by a predator [21]. Visual cues also trigger predator avoidance in bees. Bumblebees, *Bombus terrestris*, avoid flowers where cryptic predators lie in ambush [19], and it has been shown that bumblebees learn to avoid specific shapes, rather than colour contrasts [30]. Likewise, honeybees, *Apis mellifera*, use visual cues to detect predators. Although the exact features that they use to identify predators are unknown, size, colour contrast and movement affect the probability that honeybees detected cryptic crab spiders [43]. In our experiment, *N. strigata* bees responded differently to the presence of dead *O. smaragdina* and *P. dives* ants. These ants differ in a number of traits, size being the most conspicuous one. Bees may have used body size to discriminate between predatory and harmless species, but it is also possible that size acts as a non-specific feature, much as the volatile in the urine of carnivores [39], and that bigger visual stimuli, of any kind, are perceived as more threatening.

Predator avoidance has important ecological and evolutionary consequences. Predators affect the structure of food webs and ecological communities, and the indirect effects of predators, mediated by the predator-avoidance responses of their prey, are at least as strong as the direct effects, mediated by prey consumption [2,3]. To understand these indirect effects, it is important to study the behavioural response of prey to predator cues. In the system we study, the increase in the time that *N. strigata* bees spend examining flowers upon detection of *O. smaragdina* cues explains why the foraging efficiency of *N. strigata* bees is higher at ant-free than at ant-harboursing plants. This difference in foraging efficiency eventually explains why large *Xylocopa* bees, which are not susceptible to predation from *O. smaragdina* ants, preferentially forage at ant-harboursing *M. malabathricum* plants. The preference of *Xylocopa* bees, in turn, explains the higher reproductive success of plants with *O. smaragdina* nests [3].

We thank the Singapore National Parks Board for research permits. Luisa Amo de Paz, Lars Chittka, Guadalupe Corcobado and three anonymous reviewers made useful comments on previous versions of the manuscript. This work was supported by the Ministerio de Ciencia e Innovación/FEDER (projects CGL2007-63223/BOS and CGL2010-16795 to M.A.R.G.) and CSIC (studentship JAE-Pre\_08\_01008 to F.G.G.).



## References

- Lima SL, Dill LM. 1990 Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640. (doi:10.1139/z90-092)
- Peacor SD, Werner EE. 2001 The contribution of trait-mediated indirect effects to the net effects of a predator. *Proc. Natl Acad. Sci. USA* **98**, 3904–3908. (doi:10.1073/pnas.071061998)
- González FG, Santamaría L, Corlett RT, Rodríguez-Girones MA. 2013 Flowers attract weaver ants that deter less effective pollinators. *J. Ecol.* **101**, 78–85.
- Kelley JL, Magurran AE. 2003 Learned predator recognition and antipredator responses in fishes. *Fish Fish* **4**, 216–226. (doi:10.1046/j.1467-2979.2003.00126.x)
- Dicke M, Grostal P. 2001 Chemical detection of natural enemies by arthropods: an ecological perspective. *Annu. Rev. Ecol. Syst.* **32**, 1–23. (doi:10.1146/annurev.ecolsys.32.081501.113951)
- Helfman GS. 1989 Threat-sensitive predator avoidance in damselfish–trumpetfish interactions. *Behav. Ecol. Sociobiol.* **24**, 47–58. (doi:10.1007/bf00300117)
- Hartman EJ, Abrahams MV. 2000 Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proc. R. Soc. Lond. B* **267**, 571–575. (doi:10.1098/rspb.2000.1039)
- Smith ME, Belk MC. 2001 Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? *Behav. Ecol. Sociobiol.* **51**, 101–107. (doi:10.1007/s002650100415)
- Kats LB, Dill LM. 1998 The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361–394.
- Brown GE, Cowan J. 2000 Foraging trade-offs and predator inspection in an Ostariophysan fish: switching from chemical to visual cues. *Behaviour* **137**, 181–195. (doi:10.1163/1568539005020215)
- Brown GE, Magnavacca G. 2003 Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? *Ethology* **109**, 739–750. (doi:10.1046/j.1439-0310.2003.00919.x)
- Chittka L, Thomson JD. 2001 *Cognitive ecology of pollination: animal behaviour and floral evolution*. Cambridge, UK: Cambridge University Press.
- Rodríguez-Girones MA, Bosch J. 2012 Effects of body size and sociality on the anti-predator behaviour of foraging bees. *Oikos* **121**, 1473–1482. (doi:10.1111/j.1600-0706.2011.19473.x)
- Dukas R. 2001 Effects of perceived danger on flower choice by bees. *Ecol. Lett.* **4**, 327–333. (doi:10.1046/j.1461-0248.2001.00228.x)
- Brechbühl R, Casas J, Bacher S. 2009 Ineffective crypsis in a crab spider: a prey community perspective. *Proc. R. Soc. B* **7**, 739–746. (doi:10.1098/rspb.2009.1632)
- Dukas R. 2005 Bumble bee predators reduce pollinator density and plant fitness. *Ecology* **86**, 1401–1406. (doi:10.1890/04-1663)
- Dukas R, Morse DH. 2003 Crab spiders affect flower visitation by bees. *Oikos* **101**, 157–163. (doi:10.1034/j.1600-0706.2003.12143.x)
- Heiling AM, Herberstein ME. 2004 Predator-prey coevolution: Australian native bees avoid their spider predators. *Proc. R. Soc. Lond. B* **271**, S196–S198. (doi:10.1098/rsbl.2003.0138)
- Ings TC, Chittka L. 2008 Speed–accuracy tradeoffs and false alarms in bee responses to cryptic predators. *Curr. Biol.* **18**, 1520–1524. (doi:10.1016/j.cub.2008.07.074)
- Heiling AM, Herberstein ME, Chittka L. 2003 Pollinator attraction: crab-spiders manipulate flower signals. *Nature* **421**, 334–334. (doi:10.1038/421333a)
- Llandres AL, González FG, Rodríguez-Girones MA. 2013 Social but not solitary bees reject dangerous flowers where a conspecific has recently been attacked. *Anim. Behav.* **85**, 97–102. (doi:10.1016/j.anbehav.2012.10.012)
- Magurran AE, Irving PW, Henderson PA. 1996 Is there a fish alarm pheromone? A wild study and critique. *Proc. R. Soc. Lond. B* **263**, 1551–1556. (doi:10.1098/rspb.1996.0227)
- Brown JS, Laundre JW, Gurung M. 1999 The ecology of fear: optimal foraging, game theory, and trophic interactions. *J. Mammal.* **80**, 385–399. (doi:10.2307/1383287)
- Lima SL, Steury TD. 2005 Perception of predation risk: the foundation of nonlethal predator–prey interactions. In *Ecology of predator–prey interactions* (eds P Barbosa, I Castellanos), pp. 166–188. New York, NY: Oxford University Press.
- Rodríguez-Girones MA. 2012 Possible top-down control of solitary bee populations by ambush predators. *Behav. Ecol.* **23**, 559–565. (doi:10.1093/beheco/arr228)
- Clark CW, Dukas R. 1994 Balancing foraging and antipredator demands: an advantage of sociality. *Am. Nat.* **144**, 542–548. (doi:10.1086/285693)
- Goulson D, Chapman JW, Hughes WOH. 2001 Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *J. Insect Behav.* **14**, 669–678. (doi:10.1023/a:1012231419067)
- Tsuji K, Hasyim A, Harlion Nakamura K. 2004 Asian weaver ants, *Oecophylla smaragdina*, and their repelling of pollinators. *Ecol. Res.* **19**, 669–673. (doi:10.1111/j.1440-1703.2004.00682.x)
- Stout JC, Goulson D. 2001 The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Anim. Behav.* **62**, 183–189. (doi:10.1006/anbe.2001.1729)
- Ings TC, Wang MY, Chittka L. 2012 Colour-independent shape recognition of cryptic predators by bumblebees. *Behav. Ecol. Sociobiol.* **66**, 487–496. (doi:10.1007/s00265-011-1295-y)
- R Development Core Team 2010 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Barnett AG, Dobson AJ. 2008 *An introduction to generalized linear models*, 3rd edn. Boca Raton, FL: Chapman & Hall/CRC Press.
- Ferrari MCO, Vrtelova J, Brown GE, Chivers DP. 2012 Understanding the role of uncertainty on learning and retention of predator information. *Anim. Cogn.* **15**, 807–813. (doi:10.1007/s10071-012-0505-y)
- Giurfa M, Zhang SW, Jenett A, Menzel R, Srinivasan MV. 2001 The concepts of ‘sameness’ and ‘difference’ in an insect. *Nature* **410**, 930–933. (doi:10.1038/35073582)
- Dacke M, Srinivasan MV. 2008 Evidence for counting in insects. *Anim. Cogn.* **11**, 683–689. (doi:10.1007/s10071-008-0159-y)
- Carazo P, Font E, Forteza-Behrendt E, Desfilis E. 2009 Quantity discrimination in *Tenebrio molitor*: evidence of numerosity discrimination in an invertebrate? *Anim. Cogn.* **12**, 463–470. (doi:10.1007/s10071-008-0207-7)
- Chittka L, Niven J. 2009 Are bigger brains better? *Curr. Biol.* **19**, R995–R1008. (doi:10.1016/j.cub.2009.08.023)
- Mathis A, Vincent F. 2000 Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Canadian J. Zool.* **78**, 1646–1652. (doi:10.1139/cjz-78-9-1646)
- Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ, Datta SR, Spehr M, Fendt M, Liberles SD. 2011 Detection and avoidance of a carnivore odor by prey. *Proc. Natl Acad. Sci. USA* **108**, 11 235–11 240. (doi:10.1073/pnas.1103317108)
- Amo L, Lopez P, Martin J. 2006 Can wall lizards combine chemical and visual cues to discriminate predatory from non-predatory snakes inside refuges? *Ethology* **112**, 478–484. (doi:10.1111/j.1439-0310.2005.01170.x)
- Srinivasan MV, Lehrer M. 1988 Spatial acuity of honeybee vision and its spectral properties. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **162**, 159–172. (doi:10.1007/bf00606081)
- Van Mele P, Vayssières JF, Adanonon A, Sinzogan A. 2009 Ant cues affect the oviposition behaviour of fruit flies (Diptera: Tephritidae) in Africa. *Physiol. Entomol.* **34**, 256–261. (doi:10.1111/j.1365-3032.2009.00685.x)
- Llandres AL, Rodríguez-Girones MA. 2011 Spider movement, UV reflectance and size, but not spider crypsis, affect the response of honeybees to Australian crab spiders. *PLoS ONE* **6**, 11. (doi:10.1371/journal.pone.0017136)