



Floral signal complexity as a possible adaptation to environmental variability: a test using nectar-foraging bumblebees, *Bombus impatiens*

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Floral signals are typically emitted across multiple sensory modalities, although why they are multimodal is unclear. One possible explanation is that multimodal signalling ensures that at least one signal component will be transmitted effectively under varying environmental conditions (the 'efficacy backup' hypothesis). For example, by transmitting both component A and B, a signaller can communicate under environmental conditions where transmission of component A is reduced; component B 'backs up' A. To test this hypothesis, we determined whether a floral scent could back up a floral colour signal when light levels were low. We trained nectar-foraging bumblebees to discriminate rewarding and unrewarding targets that differed in colour, scent, or both colour and scent, and then presented the targets at different levels of illumination. We measured bees' accuracy at distinguishing the two targets and their rate of visits to the trained target. Performance on both measures declined under low light when targets were unscented. The presence of scent reduced the loss of accuracy under low light, supporting the efficacy backup hypothesis, but this effect depended upon the colour of the previously rewarded target. In contrast, the presence of scent did not affect the overall rate of correct visits under low light (correct visits/foraging time). A backup mechanism that maintains accuracy, but not rate of nectar collection, does not necessarily benefit the pollinator. However, it most likely benefits the plant through reduced pollen wastage. In short, multimodal floral signals may benefit the plant by improving pollen transfer, while not benefiting the pollinator.

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Flowers use a variety of signals to advertise rewards to their pollinators. These signals are often transmitted simultaneously across multiple sensory modalities, including visual, olfactory, gustatory, tactile, and even acoustic modalities (reviewed in Raguso 2004). While much is known about signal function within particular modalities, such as vision and olfaction, relatively little is known about why floral displays simultaneously emit signals in multiple modalities, despite potential production costs (e.g. metabolic costs of floral display components: Helsper et al. 1998; Galen 1999) and ecological costs (e.g. risk of attracting antagonists: Theis 2006). Multimodal signalling in flowers has been shown to enhance bumblebee nectar foraging (Kulahci et al. 2008; Leonard et al. 2011a), yet why it does so remains an open question (reviewed in Leonard et al. 2011b, c).

A number of hypotheses exist for why signals in nature are generally multimodal (Guilford & Dawkins 1991; Rowe 1999;

Candolin 2003; Hebets & Papaj 2005). One set of hypotheses is efficacy based, pertaining to how well a signal is transmitted or detected and processed by the receiver (Guilford & Dawkins 1991; Hebets & Papaj 2005). For instance, the 'efficacy backup hypothesis' (Hebets & Papaj 2005) focuses on the potential for environmental conditions to obscure signal transmission (Bradbury & Vehrencamp 1998). Overcast skies might reduce the efficacy of visual signals; similarly, windy conditions might reduce the efficacy of olfactory signals. The efficacy backup hypothesis states that components of a multimodal signal provide functional redundancy in the face of unpredictable environmental change. On windy days, when scents are less localizable, visual stimuli may allow bees to locate and identify flowers; on overcast days or in deep shade, when visual stimuli are difficult to discern, scent may be more useful.

The backup hypothesis is a plausible explanation for multimodal signalling for several reasons. Environmental conditions are known to obscure signal transmission and detection (Bradbury & Vehrencamp 1998; Candolin 2003). Additionally, such conditions change in ways that can be difficult, if not impossible, for organisms to predict. Finally, many animals possess the capacity to use different sensory modalities under different environmental conditions (e.g. Able 1991; Chittka et al. 1999; Eklöf et al. 2002; Kroder

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et al. 2007). Despite the plausibility of the backup hypothesis, it has rarely been tested. Of notable exception are recent studies of visual and vibratory signal components in wolf spider courtship displays (Rundus et al. 2010; Wilgers & Hebets 2011).

If multimodal signals serve a backup function, such signals should maintain high levels of efficacy across a range of variation in the environment. For floral signals, variation in the environment may be due to physical factors (e.g. variation in light, wind, temperature, humidity, etc.) or biotic factors (e.g. variation in floral species composition, degree of vegetative cover, etc.). Although pollinators' responses to multimodal signals have been explored in a number of studies (reviewed in Leonard et al. 2011b, c), no study has assessed the relative effectiveness of multimodal and unimodal signals under variable environmental conditions. The aim of the present study was to test the efficacy backup hypothesis in a plant–pollinator context with the goal of providing insight into the adaptive benefit of multimodal floral signals.

We specifically asked whether the olfactory component of a floral display can compensate for the loss of visual information under low illumination, as might occur at dawn or dusk, in deep shade, or under overcast skies. Reduced illumination has been found to increase search times for small flowers in bees (Chittka & Spaethe 2007), suggesting that the efficacy of visual signals is indeed reduced at low light levels.

We first trained nectar-foraging bumblebees to discriminate rewarding and unrewarding targets that differed in colour, scent, or both colour and scent. We predicted that visual signals would lose efficacy as light levels declined, resulting in reduced performance, but that this decline in performance would be mitigated by the addition of scent.

Our measures of bee performance included accuracy in landing on the previously rewarded target type (i.e. correct landings over total landings) and rate of visits to the previously rewarded type (i.e. correct landings per foraging time). These performance measures not only have implications for pollinator fitness (rate of energy intake), but also for plant fitness (rate of pollen transfer to or from conspecifics). Our design thus allowed us to consider the benefit of a multimodal signal from the perspectives of both the sender (plant) and receiver (pollinator).

METHODS

Two commercially obtained colonies of the common eastern bumblebee, *Bombus impatiens* (Koppert Biological Systems, Romulus, MI, U.S.A.), were used ($N = 63$ and 33 from the different colonies). During the experiment, a single colony was connected to a foraging arena ($86.4 \times 76.2 \times 45.7$ cm), with plastic and mesh tubing. Gates in the tubing permitted regulation of bee traffic to the arena. Colonies were given full access to the foraging arena when trials were not occurring, where bees were fed daily a 30% (wt/wt) sucrose solution from a multiwell feeder.

Pretraining

We ran weekly pretraining sessions to identify individual bees willing to feed from the artificial flower array. During pretraining, all bees had access to a horizontal array of 60 artificial flowers (in a 10×6 grid) spaced at 8 cm intervals, an arrangement that matched arrays in experimental trials. All pretraining flowers were light blue, matte-laminated paper disks (25 mm diameter), attached to a reservoir tube containing a thin cotton wick that protruded just above the centre of the disk. To provide a situation similar to experimental trials, half of the pretraining flowers were rewarding (wick provided 30% sucrose solution), while the other half were unrewarding (dry). Rewarding and unrewarding flowers were

positioned randomly into the array. Bees that fed from artificial flowers during pretraining were tagged with an identifying number (E. H. Thorne Ltd, Wragby, U.K.) and used in further experiments.

Training

To test the backup efficacy hypothesis, bees were trained in one of two discrimination tasks: 'visual only' (targets differed in colour only) and 'bimodal' (targets differed both in colour and in scent). To determine the efficacy of the odours used in the bimodal treatment, a third group of bees were trained in an olfactory-only discrimination task (targets differed in scent only).

For the visual-only task, we used two colours that differed slightly in blue to green ratio (one with an equal ratio, Blue 1; the other with slightly more green, Blue 2). Very similar colours were chosen to make the discrimination task more challenging, increasing the chances that an additional cue would enhance discrimination. Colours were printed on waterproof paper (National Geographic, Margate, FL, U.S.A.) using a Canon Pixma MX860 inkjet printer, and laminated (Xyron matte laminate, Scottsdale, AZ, U.S.A.). We measured the reflectance of these colours (Fig. 1) and determined that the difference in chromatic contrast (colour distance) was relatively low (approximately 0.03; using spectral sensitivity functions (Peitsch et al. 1992; Stavenga et al. 1993) based on *B. impatiens* photoreceptor peak sensitivities (Skorupski & Chittka 2011) and spectrometric analysis software (AVICOL 4.0; Gomez 2006) based on calculations from Chittka 1992). A colour distance of 0.062 is relatively difficult for bumblebees (*B. terrestris*) to discriminate (Dyer & Chittka 2004).

For the bimodal task, we used the same colours but added linalool and geraniol, two structurally similar monoterpenes common in floral scents (Schiestl 2010). We pipetted 20 μ l of a 1:10 (1 M) solution of the compound in mineral oil onto a cotton swab placed inside of the pipette tip serving as the base of the target. The scents percolated through small holes in the laminated paper target. Blue 1 was always paired with linalool and Blue 2 was always paired with geraniol.

For the olfactory-only task, we again used linalool and geraniol, but all targets were Blue 1.

In training sessions, bees were released individually into the arena containing a 60-target horizontal array. For each bee, one target type was rewarding (5 μ l of 50% sucrose) and one was unrewarding (5 μ l of water). This relationship was balanced across bees.

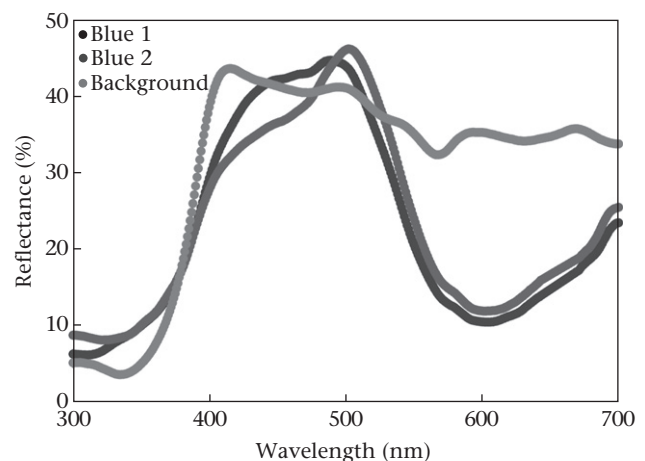


Figure 1. Percentage reflectance across wavelengths for the two colours used to distinguish floral types, Blue 1 and Blue 2, and the background. Raw data spectra were interpolated and smoothed in Avicol 4.0.

Training targets were 16 mm in diameter (the approximate size associated with increased search times under low light; Chittka & Spaethe 2007) and lacked a wick; solutions were pipetted directly onto the target. On each training trial, bees were allowed to forage in the arena for at least 10 min, or until they returned to the nest or stopped foraging for longer than 2 min, whichever occurred earlier. After each trial the bee was returned to the nest. Each bee was given at least two training trials, and as many additional trials as was necessary for the bee to reach 150 visits (mean \pm SE number of training trials: bimodal treatment: 3.1 ± 0.2 ; visual-only treatment: 2.6 ± 0.1 ; olfactory-only treatment: 2.9 ± 0.2). Between trials, we cleaned targets with 30% ethanol to remove any scent marks and redistributed their positions using one of three random patterns to prevent learning of spatial cues. All trials were conducted under 'moderate' light intensity (see below) and videorecorded.

Testing

After training (at least two trials and >150 flower visits), the foraging success of each bee was immediately assessed upon its return to the arena using an array of unrewarded (water-only) targets of the same types to which it had been trained. Whereas training occurred for all bees under 'moderate' light intensity, a bee was tested under one of three levels of light intensity (high, moderate, or low). Light intensity was manipulated by means of neutral density filters (Rosco E-colour no. 209, Stamford, CT, U.S.A.) fitted across four 250 W halogen light sources. Each filter was of optical density equal to 0.3, with approximately 50% transmittance; we layered filters to create different light levels (no filter for 'high', one filter for 'moderate' and four filters for 'low' light intensity; irradiance spectra in Fig. 2). These filter combinations produced a gradient of light levels (mean of multiple readings in five areas of arena \pm SE: low = 62 ± 3 lx, moderate = 574 ± 29 lx, high = 1342 ± 79 lx). The low level was equivalent to deep shade or thick cloud cover (around 100 lx; Chittka & Spaethe 2007); this is above those light levels at which bees switch to achromatic vision (Menzel 1981; Rose & Menzel 1981). The high light level was equivalent to light shade or slightly overcast conditions (Johnsen et al. 2006). The irradiance differences only slightly altered the position of targets in bumblebee colour space (Fig. 3) and chromatic contrast between the two colours varied little with light intensity (high: 0.0305; moderate: 0.0308; low: 0.0310). Temperature with testing lights on remained within 2 °C from starting temperature in all treatments.

Twelve bees were tested per light intensity in the visual-only and bimodal treatments, while eight bees were tested per light

intensity in the olfactory-only treatment, for a total of 96 bees. Test trials lasted for 10 min or until the bee returned to the nest (mean \pm SE number of visits per test trial = 83.7 ± 3.4). We calculated accuracy (proportion of visits to previously rewarded target type (number of correct visits/number of total visits)) and the rate of correct visits (number of visits to previously rewarded target type per time spent foraging (number of correct visits/s)). Test bees were later sacrificed for measurements of body size (thorax width), which is known to affect bumblebee foraging performance (Spaethe & Weidenmüller 2002), particularly under different light conditions (Kapustjanskij et al. 2007).

Analysis of Training Performance

We were also interested in comparing how readily bees learned to discriminate the two target types depending on the colours or odours available during training. As an index of learning performance, we estimated the number of visits it took each bee to reach a criterion of 8 out of 10 consecutive visits to the rewarding flower type. We analysed the number of visits needed to reach this criterion (square root transformed) using multiple general linear models (GLM; JMP 8.0, SAS Institute, Cary, NC, U.S.A.) and Tukey's HSD for post hoc tests ($\alpha = 0.05$). We also investigated whether there was an initial bias for bees to land on a particular colour (in visual only and bimodal) or odour (in olfactory only) on their first landing of the first training trial (chi-square tests; Microsoft Excel 2007).

Analysis of Testing Performance

Our central question of whether olfactory signals can compensate for the loss of visual information was assessed by comparing bee performance in the visual-only and bimodal treatments. The olfactory-only treatment only served as a control to determine whether the efficacy of an olfactory signal might itself change under different light intensities. Therefore, we ran two separate, targeted analyses (GLM; JMP 8.0), one for visual versus bimodal treatments to specifically test the efficacy backup hypothesis, and one for the olfactory treatment only; in each case, the outcome variables were accuracy (arcsine root transformed) and the rate of correct visits. The analysis of visual-only and bimodal assays included treatment, light intensity and colour of the previously rewarded target type (and all possible interactions) as factors in the model. The analysis of the olfactory-only assays included light intensity and odour of the previously rewarded target type (and their interaction) as factors in the model. There were no significant effects of colony (accuracy: $F_{1,94} = 0.09$, $P = 0.76$; rate of correct visits: $F_{1,94} = 0.965$, $P = 0.328$) or body size (accuracy: $F_{1,93} = 0.08$, $P = 0.783$; rate of correct visits: $F_{1,93} = 0.715$, $P = 0.400$), so these factors were not included in the model. Post hoc tests (Tukey HSD at $\alpha = 0.05$) were used for comparisons of interest.

Because there were significant colour effects in both training and testing (see Results below), we reanalysed test performance (accuracy and rate of correct visits) for the visual-only versus bimodal treatments, but with data separated by the colour of the previously rewarded target type. Modality treatment, light intensity and their interaction were the factors included in the separate models (GLM; JMP 8.0).

RESULTS

Training Performance

Bees learned to discriminate between targets more quickly when they differed in both colour and scent than when they differed in colour alone (Fig. 4). Over the course of the training

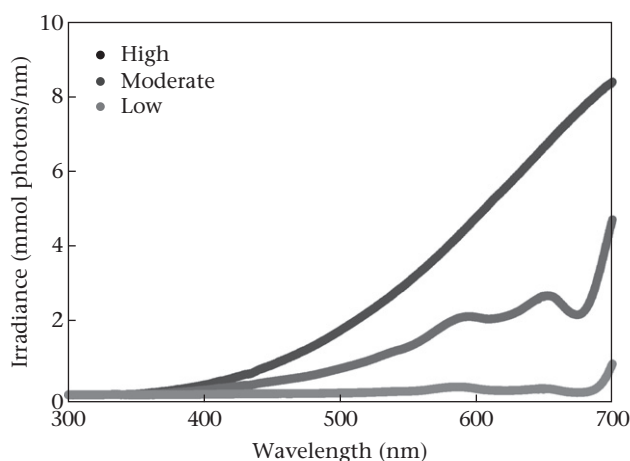


Figure 2. Total irradiance (mmol photons/nm) across wavelengths for the different light intensities.

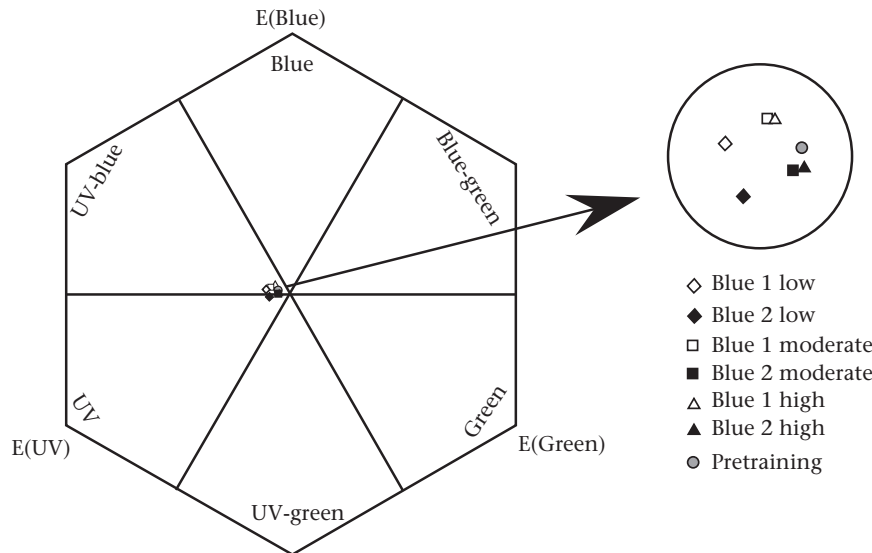


Figure 3. Location in bee colour space of each of the two experimental colours, Blue 1 and Blue 2, under three light intensities. The colour of the pretraining target under the light conditions used is also included. Inset shows a close-up of the data points. Blue 1 is denoted by white symbols, Blue 2 by black symbols, and pretraining by a grey symbol. The two colours under the same light intensity have the same symbol shape. The centre of the hexagon represents the background.

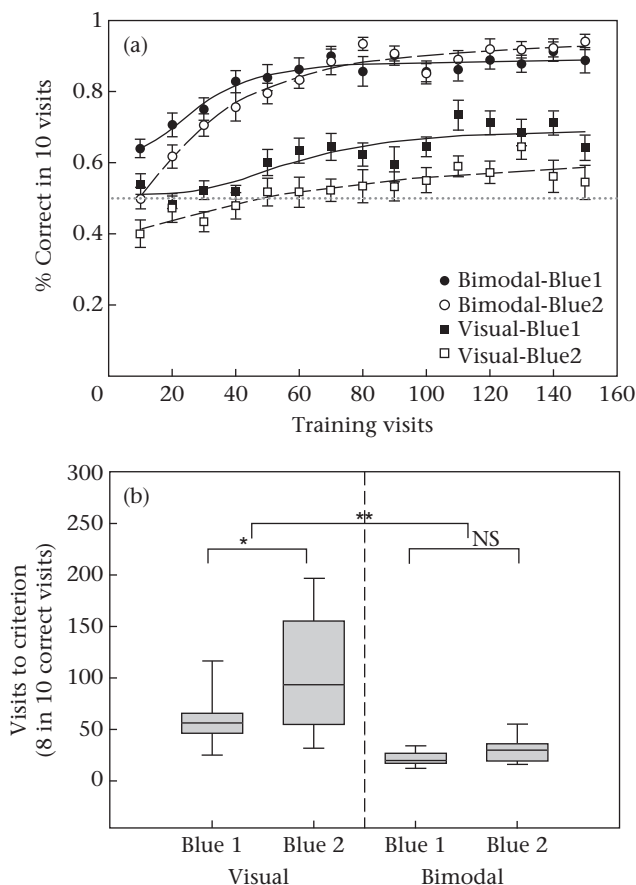


Figure 4. Performance of bees during training. (a) Learning acquisition associated with each training colour for visual and bimodal treatments. Each point represents the proportion of correct visits in 10 consecutive training visits (mean \pm SE, $N = 18$ per line). Filled symbols and solid lines denote Blue 1, unfilled symbols and dashed lines denote Blue 2. The grey dotted line denotes random choice. A four-parameter logistic curve was used to fit the data. (b) Distribution of the number of visits it took for bees to reach a criterion of 8 correct visits in 10 consecutive visits when either Blue 1 or Blue 2 was the rewarding colour in both visual and bimodal treatments ($N = 18$ bees per box plot). * $P < 0.05$; ** $P < 0.0001$.

sessions, average bee accuracy improved faster and remained higher in the bimodal than in the visual-only treatment (Fig. 4a). Bees also required significantly more visits to reach a criterion of 8 in 10 correct landings when signals were visual only than when signals were bimodal (GLM: treatment effect: $F_{1,68} = 62.14$, $P < 0.001$). There was a significant effect of training colour: bees required significantly fewer visits to reach this criterion when the colour of the rewarding target type was Blue 1 compared to Blue 2 (GLM: colour: $F_{1,68} = 9.82$, $P = 0.003$). However, the training colour effect was only significant ($P < 0.05$) in the visual-only treatment, not the bimodal treatment (Fig. 4b), although there was no significant interaction effect (GLM: treatment*colour: $F_{1,68} = 2.26$, $P = 0.137$). There was also a significant first-choice bias to land on Blue 1-coloured targets in both treatments (chi-square test: $P < 0.001$; visual only: $\chi^2_1 = 13.4$; bimodal: $\chi^2_1 = 7.1$).

Testing Performance: Visual Only versus Bimodal

An initial analysis that pooled all data from the visual-only and bimodal treatments determined that there were significant colour effects for both performance measures (GLM: effect of colour on accuracy: $F_{1,60} = 13.03$, $P < 0.001$; effect of colour on correct visit rate: $F_{1,60} = 5.95$, $P = 0.018$): performance was significantly better when the training colour was Blue 1. Given that these colour effects were also present during training (as noted above), we proceeded with separate analyses, comparing the Blue 1 visual-only group with the Blue 1/linalool bimodal group and the Blue 2 visual-only group with the Blue 2/geraniol bimodal group.

Accuracy

Bee accuracy was high for both modality treatments and across all light levels, regardless of training colour (all bees performed significantly better than random choice, 0.5, $P < 0.01$, except in the visual-only treatment with Blue 2 as the rewarding target under low light, $P = 0.056$; one sample, two-tailed t tests; see Fig. 5). Light intensity significantly affected bee accuracy with both training colours (GLM: Blue 1: $F_{2,30} = 6.902$, $P = 0.003$; Blue 2: $F_{2,30} = 7.590$, $P = 0.002$), although significant increases in accuracy with increasing light intensity were found only in the visual-only

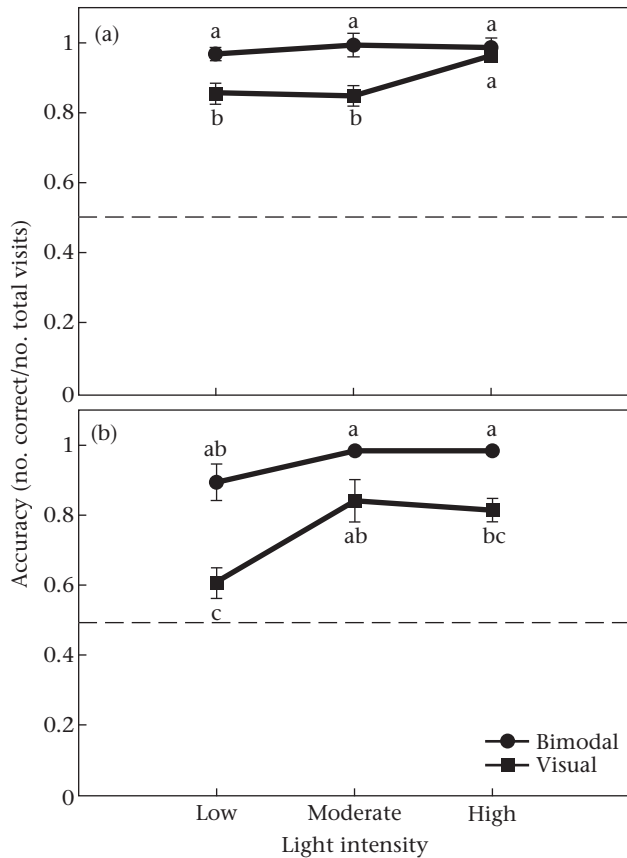


Figure 5. Accuracy of choice (number correct/number of total visits) across light intensities for visual and bimodal treatments, separated by training colour (mean \pm SE, $N = 6$ per data point). The dashed line represents random choice. Letters represent significance: points that do not share a letter were significantly different from each other at $P < 0.05$. (a) Bees that had Blue 1 as the previously rewarded target colour. (b) Bees that had Blue 2 as the previously rewarded target colour.

treatment with no significant differences across light intensities in the bimodal treatment (see Fig. 5).

When Blue 2 was the previously rewarded colour, accuracy was significantly higher when signals were bimodal compared to visual only (GLM: treatment effect: $F_{1,30} = 19.17$, $P < 0.001$; Fig. 5b). However, this was the case across all light levels; there was no significant interaction between treatment and light intensity (GLM: treatment*light intensity: $F_{2,30} = 0.701$, $P = 0.504$) and, thus, no statistical support for the efficacy backup hypothesis. When Blue 1 was the previously rewarded colour, accuracy was again significantly higher when signals were bimodal versus visual only (GLM: treatment effect: $F_{1,30} = 15.41$, $P = 0.001$). However, there was also a highly significant interaction between treatment and light level (GLM: treatment*light intensity: $F_{2,30} = 6.04$, $P = 0.006$; Fig. 5a). The pattern of interaction provided support for the efficacy backup hypothesis: there was a greater decline in accuracy with decreasing light intensity when signals were visual only compared to when they were bimodal (compare lines in Fig. 5a). In short, in terms of accuracy there is statistical support for the efficacy backup hypothesis when Blue 1 was the previously rewarded colour but not when Blue 2 was the previously rewarded colour.

Correct Visit Rate

For both training colours, the rate of visits to the previously rewarded target type (= correct visits per time) was significantly affected by light intensity (GLM: Blue 1: $F_{2,30} = 16.26$, $P < 0.001$;

Blue 2: $F_{2,30} = 15.11$, $P < 0.001$; Fig. 6). Correct visit rate significantly increased ($P < 0.05$) with increasing light intensity in both the visual-only and bimodal treatments.

When Blue 2 was the previously rewarded colour, there was no significant difference between the modality treatments (GLM: $F_{1,30} = 0.26$, $P = 0.613$; Fig. 6b) and no interaction between treatment and light intensity (GLM: treatment*light intensity: $F_{2,30} = 0.28$, $P = 0.756$), thus, in terms of correct visit rate, no statistical support for the efficacy backup hypothesis when Blue 2 was the previously rewarded colour. When Blue 1 was the previously rewarded colour, there was also no significant difference between the modality treatments overall (GLM: $F_{1,30} = 0.33$, $P = 0.568$; Fig. 6a), but there was a significant interaction between treatment and light level (GLM: treatment*light intensity: $F_{2,30} = 4.29$, $P = 0.023$). However, this pattern of interaction did not provide support for the efficacy backup hypothesis. Instead, it reflects a high rate of correct visits for the bimodal treatment under moderate light intensity (Fig. 6a, grey solid line). In other words, in terms of visit rate, there was no indication that adding odour to the discrimination task compensated for a loss of efficacy of the visual stimulus at low light levels.

Olfactory-only Assays

The olfactory-only assays served as a control to determine whether the efficacy of olfactory signals was affected by different

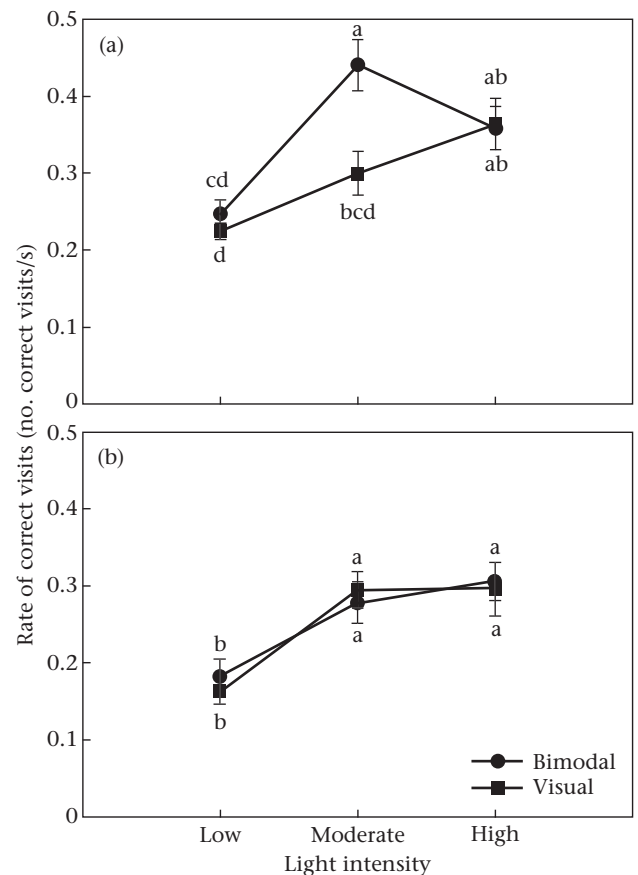


Figure 6. Correct visit rate (number of correct visits/s) across light intensities for visual and bimodal treatments, separated by training colour (mean \pm SE, $N = 6$ per data point). Letters represent significance: points that do not share a letter were significantly different from each other at $P < 0.05$. (a) Bees that had Blue 1 as the previously rewarded target colour. (b) Bees that had Blue 2 as the previously rewarded target colour.

light intensities. We noted that during training, bees did not show a first-choice bias for a particular odour (chi-square test: $\chi^2_1 = 1.50$, $P = 0.121$), nor was their discrimination between targets affected by the identity of the previously rewarded odour: there was no significant difference in the number of visits to reach a criterion of 8 in 10 correct (GLM: odour effect: $F_{1,34} = 0.20$, $P = 0.659$). During testing, bee accuracy at distinguishing the previously rewarded target type when signals were olfactory only (Fig. 7a) was not significantly affected by light intensity (GLM: $F_{2,18} = 0.24$, $P = 0.789$), odour type (GLM: $F_{2,18} = 0.39$, $P = 0.543$), or their interaction (GLM: $F_{2,18} = 1.41$, $P = 0.269$). However, the rate at which bees visited previously rewarded targets (Fig. 7b) was significantly affected by light intensity (GLM: $F_{2,18} = 3.60$, $P = 0.049$), with lower rates overall at low light intensity than at moderate or high intensity (although rates at high and moderate intensity were not significantly different). Correct visit rate was not affected by type of odour (GLM: $F_{2,18} = 0.53$, $P = 0.476$) or the interaction of light intensity and odour type (GLM: $F_{2,18} = 2.67$, $P = 0.097$).

DISCUSSION

Environmental heterogeneity is a major, yet largely overlooked, factor in plant–pollinator communication (but see Kilkenny & Galloway 2008). Factors such as illumination (Jakobsen & Olsen 1994; Chittka & Spaethe 2007; Kapustjanskij et al. 2007), wind speed (Streinzer et al. 2009) and temperature (Jakobsen & Olsen

1994; Sagae et al. 2008) can all affect the transmission and/or perception of floral signals. The efficacy backup hypothesis accounts for potential environmental heterogeneity, proposing that multimodal signals ensure that pollinators receive appropriate floral information despite environmental degradation of signalling in one modality. Our experiment directly assessed how bumblebees' ability to discriminate between floral types that differed in reward value depended both upon light level and upon the multimodal nature of the floral display. We first established that low illumination reduced the efficacy of visual-only signals, both in terms of landing accuracy (Fig. 5) and correct visit rate (Fig. 6), regardless of training colour. Then we asked whether the visual signal's loss of efficacy under low light was reduced with the addition of an olfactory component (bimodal targets). We found support for this idea in terms of landing accuracy; however, the effect was conditional upon training colour, occurring only when bees were rewarded for visiting the colour they initially preferred (Blue 1). The observed increase in accuracy is most likely due to additional information provided by scent, given that bees were highly accurate in discriminating flowers based solely on these scents alone in the olfactory-only treatment. However, another possible mechanism could be that the presence of scent improved colour perception (Kunze & Gumbert 2001; Leonard et al. 2011a). Assessing the underlying mechanism behind the context-dependent backup effect on accuracy is an intriguing question for future research.

When we considered correct visit rate, a measure that takes into account not only accuracy but also foraging speed, we found no evidence that an olfactory signal compensated for the loss of visual information: a similar decrease in correct visit rate under low light occurred when the signals were visual, bimodal or olfactory (Fig. 6: visual versus bimodal; Fig. 7b: olfactory only). In other words, bees were making fewer correct choices per unit time when illumination was low, regardless of whether an olfactory component was present. The effect of light intensity on correct visit rate suggests that light intensity affects not only discrimination of visual cues but also the speed at which a bee forages for nectar, an effect previously demonstrated in honeybee foraging (Rose & Menzel 1981). Thus, because the correct visit rate remained low under low light intensity even with olfactory information, the presence of scent could not compensate for the loss of visual efficacy in terms of reward collection for the bee. In short, our results point towards a complex, conditional relationship among signal complexity, pollinator behaviour and components of plant and pollinator fitness.

Although many studies have explored the effect of multimodal floral signals on bees (e.g. Couvillon & Bitterman 1980, 1982, 1987; Giurfa et al. 1994; Kunze & Gumbert 2001; Reinhard et al. 2004; Leonard et al. 2011a), efficacy backup was not a focus of these studies. Our results show that efficacy backup is a potential explanation for the evolution of multimodal floral signals, but this is not mutually exclusive with other hypotheses that propose differing explanations (Leonard et al. 2011c). Content-based hypotheses relate to the information provided by the signaller (plant), whereas efficacy-based hypotheses relate to the ability of the receiver (pollinator) to detect and process that information (Hebets & Papaj 2005). For example, the 'efficacy trade-off hypothesis' proposes that different modalities deal with different challenges of signal transmission through the environment (e.g. short-range versus long-range; Streinzer et al. 2009). The 'attention-altering hypothesis' suggests that a signal in one modality alters the attention of the receiver to a signal in another modality (e.g. the presence of scent improves colour perception; Kunze & Gumbert 2001; Leonard et al. 2011a). Multiple explanations could be relevant under different circumstances or with different organisms.

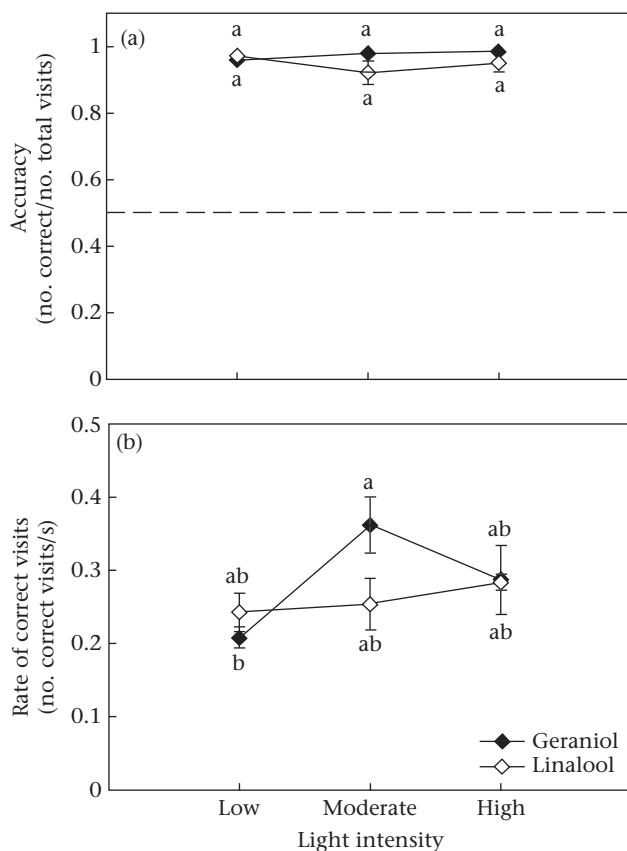


Figure 7. Bee performance for the two scents, geraniol and linalool, used in the olfactory-only treatment across different light intensities (mean \pm SE, $N = 8$ per data point). Letters represent significance: points that do not share a letter were significantly different from each other at $P < 0.05$. (a) Bee accuracy (number correct/number of total visits). The dashed line represents random choice. (b) Correct visit rate (number of correct visits/s).

Conditional Effects of Signal Types on Bee Behaviour

Why did the presence of an olfactory signal back up the loss of visual information (in terms of landing accuracy) when the colour rewarded during training was Blue 1, but not Blue 2? The colours used in this experiment were most likely perceived by the bees to be very similar, based on their close location in bee colour space (Fig. 3). It is thus intriguing that we found a significant initial landing preference, as well as learning and performance differences based on training colour. Even though half our subjects learned to visit Blue 2 during training sessions (based upon our learning criterion), an innate bias towards Blue 1 could have affected their test performance. We noted that Blue 1 had greater reflectance at lower wavelengths (blue region; Fig. 1), and that it was both initially preferred and learned more readily. Perhaps the bees' stronger innate preference for lower wavelengths (Giurfa et al. 1995; Gumbert 2000; Chittka et al. 2001; Raine & Chittka 2007) resulted in faster learning or stronger persistence when Blue 1 was rewarding (cf. Giurfa et al. 1997; Ings et al. 2009). It seems unlikely that the bias towards Blue 1 resulted from exposure to the light blue used in pretraining trials since that colour appears to be closer to Blue 2 in bee colour space (Fig. 3).

Whatever the source of the bias for Blue 1, its presence constrains the generality of the 'backup' effect of floral scent on landing accuracy. For example, bees trained to land on Blue 2 still landed on Blue 1 during tests at a relatively high frequency (Fig. 5b), across all three light levels. Thus, for Blue 2-trained bees, the relative benefit of a bimodal target did not increase as light levels declined, but stayed relatively constant: in contrast to Blue 1-trained bees, Blue 2-trained bees benefited from a bimodal target even when illumination was high. These findings suggest interplay between the relative attractiveness of visual versus olfactory signals and their benefit in a changing environment. Essentially, if a flower presents a highly attractive visual signal, the benefits of multimodality only become clear with the loss of visual information. For flowers with less attractive visual signals, multimodality is evidently advantageous across all light levels. This idea could be tested by systematically varying the attractiveness of visual and olfactory signals, while measuring bee foraging performance along an environmental gradient (e.g. illumination levels, wind speed).

This study utilized both similar colours and similar scents known to be relatively attractive for the bee, but we might expect different results with different colours, scents or colour–scent combinations, especially given the context dependence observed with the colours used in this study. For example, olfactory information may not be very useful if the visual signal is highly divergent (Giurfa et al. 1994) because the colours are likely to be discriminable even under low light. In this case, additional olfactory information would be less likely to increase bee performance. On the other hand, olfactory information may be even more useful if the scents are more divergent (or otherwise more easily distinguishable) and more likely to increase bee performance, especially if the colours are more similar. Given our results, we might also expect a greater benefit of multimodality with the presence of a less favoured stimulus. Our goal was not to determine under which circumstances efficacy backup is likely to be relevant, but to show that efficacy backup can explain evolution of multimodal signals, which is supported by our results.

Potential Effects of Signal Complexity on Bee versus Plant Fitness

We considered two related measures of bee foraging behaviour under different light levels. The first of these was landing accuracy, as discussed above. The second was visit rate to the previously rewarded flower type. Although we found that bees trained and

tested with bimodal targets showed a higher landing accuracy than bees whose targets were visual-only, they did not show a higher rate of landing upon the previously rewarded flower type. Accuracy is commonly used as the only outcome variable in learning experiments. Although it may be relevant when bees face significant costs associated with incorrect choices (time costs (Burns 2005), which may depend upon the frequency of rewarding flowers in a community (Burns & Dyer 2008), or nutritive costs (Adler 2000), which may depend upon the frequency of unfavourable flowering species in a community), it may not always be the most appropriate measure of pollinator fitness (Burns 2005). Instead, nectar collection rate, or a related variable such as correct visit rate, is more likely to have the most direct impact on bee fitness (Pelletier & McNeil 2003). In contrast, bee accuracy may have a particularly strong impact on plant fitness because it facilitates pollen transfer among conspecific plants.

Accuracy may be at a premium for plants when illumination declines, as shade-growing plants may receive fewer pollinator visits than their counterparts growing in areas of high irradiance (Herrera 1995b; O'Connell & Johnson 1998; Kilkenny & Galloway 2008; but see Sánchez-Lafuente et al. 2005; Hansen & Totland 2006). For plants that face this kind of spatial variation in light levels, differences in pollinator visits have been attributed to the effects of shade on floral display size, as well as to accompanying decreases in temperature, relevant for ectothermic foragers like bees (Herrera 1995a, b, 1997). Our experiment suggests a third explanation for bees' reluctance to visit shade-growing plants: they also forage less effectively under reduced light levels. The observed reduction in foraging speed at low light levels occurred without significant changes in temperature ($\pm 2^\circ\text{C}$ with or without lights on), which we monitored throughout the experiment.

For plants that face temporal variation in light levels (e.g. occasional cloudy days), our results show a benefit of transmitting scent (at least when flowers are visually difficult to detect). One might even expect that plants would increase scent production when visual cues lose efficacy. Do plants do this? We found no evidence of this in the literature. In fact, several studies suggest that plants reduce their emission of floral volatiles when illumination is decreased (Jakobsen & Olsen 1994; Jakobsen et al. 1994; Underwood et al. 2005). This finding suggests a signalling strategy different from an efficacy backup strategy: rather than wasting energy to produce scents for pollinators that may be reluctant to forage under low light levels, the plant may conserve resources for signalling until environmental conditions are better suited for pollinator attraction.

Finally, variation in light levels is only one of multiple aspects of environmental heterogeneity potentially relevant to floral displays. For example, although we focused on the potential for an olfactory signal to compensate for the loss of visual information, the complementary relationship is possible: perhaps components of the visual display compensate for olfactory 'uncertainty', which may occur when wind speed increases, when wind becomes more turbulent, or when volatiles from coflowering plant species are more similar and thus more difficult to discriminate. We plan to explore these possibilities in future experiments.

Conclusion

Although interest in understanding the function of complex multimodal signals has grown in the past decade (reviewed in Hebets & Papaj 2005), few studies have explored this paradigm in a plant–pollinator system (Leonard et al. 2011b). Taken as a whole, our results suggest an adaptive benefit of multimodal floral signals may be to maintain pollinators' accuracy under environmental variability. Our results show support for the efficacy backup hypothesis in terms of accuracy, but not in the rate of correct visits.

Interestingly, the backup function appears to be context dependent, given that backup only occurred when one of the two colours used was rewarding.

Since plants may benefit directly from pollinator accuracy while pollinators benefit directly from their rate of correct visits, plants may benefit more from a backup function of multimodal signals than do pollinators. While we focused on the potential benefit of a multimodal floral signal under changing environmental conditions, many other possibilities remain to be explored in more detail (Leonard et al. 2011c), including the potential for a multimodal floral signal to facilitate discrimination learning (Kulahci et al. 2008; Leonard et al. 2011a), promote floral constancy (Gegeer 2005), or facilitate pollinators' ability to locate a flower at different spatial scales (Streinzer et al. 2009).

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