ORIGINAL RESEARCH

Bumblebees require visual pollen stimuli to initiate and multimodal stimuli to complete a full behavioral sequence in close-range flower orientation

Saskia Wilmsen1 | Robin Gottlieb1 | Robert R. Junker1,2 | Klaus Lunau1

Abstract
Flower visits are complex encounters, in which animals are attracted by floral signals, guided toward the site of the first physical contact with a flower, land, and finally take up floral rewards. At close range, signals of stamens and pollen play an important role to facilitate flower handling in bees, yet the pollen stimuli eliciting behavioral responses are poorly known. In this study, we test the response of flower-naive bumblebees (Bombus terrestris) toward single and multimodal pollen stimuli as compared to natural dandelion pollen. As artificial pollen stimuli, we used the yellow flavonoid pigment quercetin, the scent compound eugenol, the amino acid proline, the monosaccharide glucose, and the texture of pollen-grain-sized glass pellets as a tactile stimulus. Three test stimuli, dandelion pollen, one out of various uni- and multimodal stimulus combinations, and a solvent control were presented simultaneously to individual bumblebees, whose response was recorded. The results indicate that bumblebees respond in an irreversible sequence of behavioral reactions. Bumblebees approached the visual stimulus quercetin as often as natural dandelion pollen. An additional olfactory stimulus resulted in slightly more frequent landings. The multimodal stimulus combinations including visual, olfactory, gustatory, and tactile stimuli elicited approaches, antennal contacts, and landings as often as natural pollen. Subsequent reactions like proboscis extension, mandible biting, and buzzing were more often but not regularly observed at dandelion pollen. Our study shows that visual signals of pollen are sufficient to trigger initial responses of bumblebees, whereas multimodal pollen stimuli elicit full behavioral response as compared to natural pollen. Our results suggest a major role of pollen cues for the attraction of bees toward flowers and also explain, why many floral guides mimic the visual signals of pollen and anthers, that is, the yellow and UV-absorbing color, to direct bumblebees toward the site where they access the floral rewards.

KEYWORDS
Bombus terrestris, bumblebee, flower recognition, multimodal stimuli, pollen, stamen mimicry
Many flowering plants depend on insects as pollinators to secure reproduction, while offering nectar, pollen, and other resources as primary rewards. Flowers are sensory billboards (Raguso, 2004) displaying multimodal signals (Junker & Parachnowitsch, 2015) including visual signals, for example, shape, symmetry, color patterns, colors and contrasts as visual signals (Dafri & Kevan, 1996; Giurfa et al., 1999; Lehrer, Horridge, Zhang, & Gadagkar, 1995; Lunau, 2011; Osche, 1983), olfactory signals (Raguso, 2009), tactile cues (Kevan & Lane, 1985), and gustatory stimuli (Lipp, 1991; Willmer, 2011). Flowers attract pollinators from a distance, guide them at close range toward a distinct site of the flower, trigger the landing on the flower, and facilitate finding the floral resource while transferring pollen from the pollinator to the stigma and from the flower to the pollinators’ body (Lunau, 1991, 1992). Whereas colored bracts or petals are mostly large-sized to attract flower visitors from some distance, color patterns of the petals and other flower organs are small and more important for orientation at close range. This visual color pattern is paralleled by an olfactory, gustatory and tactile pattern (Burdon, Raguso, Kessler, & Parachnowitsch, 2015). Studies of flower odor chemistry suggest that nectar and pollen rewards frequently differ in the quantity and composition of volatile compounds compared to other floral tissues, and that such within-flower spatial variation may function as odor guides to direct pollinators to nectar (Dötterl & Jürgens, 2005) or pollen rewards (Bergström et al., 1995; Dobson, Danielson, & Van Wesep, 1999). Stamens, that is, anthers and pollen, take over important parts of floral signaling (Lunau, 2000) when offering visually, chemically and tactically conspicuous pollen that many flower visitors can eat or collect.

Female bees need at least two floral resources, nectar for individual energy supply and pollen for egg maturation (Cane, 2016) and the provision of their offspring (Alford, 1975; Roulston & Cane, 2000). The bees' response to various stimuli associated with nectar including color (Zhang, Larson-Rabin, Li, & Wang, 2012), glistening (Endress & Steiner, 1996), scent (Howell & Alarcon, 2007), and taste (Frost, Shutler, & Hillier, 2012; Gil, Menzel, & De Marco, 2008; Haupt, 2004; Menzel, 1993) have been extensively studied, whereas similar responses to pollen are quite little studied. Contrary to nectar, remote sensing of pollen by means of visual signals, mostly caused by yellow pigments, are important cues for pollen-eating and pollen-collecting insects (Lunau, 2000, 2006). Comparable to nectar, pollen contains chemical cues emitted from the pollenkitt of bee-pollinated flowers (Dobson, 1988; Dobson & Bergström, 2000; Stanley & Linskens, 1974), but their role for identification of pollen as such is not known. Pollenkitt is a sticky pollen coat material produced by the anther tapetum of most angiosperms (Pacini & Hesse, 2005) and produces the relevant visual and chemical cues of pollen for pollinators. Differently from nectar, it seems impossible for bees to sense the relevant nutrients of pollen, proteins, until the pollen grains have been crushed with the mandibles (Lunau, Ploerek, Krohn, & Pacini, 2015). Moreover, sensing of proteins by means of insect taste receptors is not known (De Brito Sanchez, 2011; Rüdenauer, Späthe, & Leonhardt, 2015).
pollen-grain-sized glass pellets were taken as a stimulus. The glass pellets of a diameter 40–80 μm match the size of common pollen grains of entomophilous flowers (Harder & Thomson, 1989). In this study, we explicitly tested whether pollen displays visual, olfactory, gustatory, and tactile key stimuli that enable bees to detect and identify pollen.

To analyze the behavioral sequence of the tested bumblebee workers to the presented stimuli, the experiments were recorded by a high-speed camera and analyzed according to distinct behavioral reactions which were: approach, antennal contact, landing, proboscis extension, buzzing and biting with mandibles. Thus, by using an experimental setup, we provide insights into how bumblebees respond to uni- and multimodal pollen stimuli, which is a first step toward an understanding of pollen as salient objects and not only as nutritious reward.

The experiments were carried out at variable room temperature from July 2012 to March 2014 in an indoor bumblebee laboratory at the Institute of Sensory Ecology of the Heinrich-Heine-University Düsseldorf. According to the bumblebees activity, more experiments were conducted in the morning than in the afternoon. For behavioral experiments, we used queenright colonies of Bombus terrestris (re-natur GmbH, Ruhwinkel, Germany) with approximately 20–40 individual workers. The hive was connected by a corridor to a general flight cage. The experiment was terminated if the bumblebee did not respond within 5 min; non-responding bumblebees were excluded from the analysis. The artificial flower was changed and a new trial was started with another bumblebee.

### 2.2 Test stimuli

Artificial flowers consisted of a circular filter paper (Rundfilter NM 615, Ø 70 mm, Macherey-Nagel, Düren, Germany) to which three test stimuli were attached. Before each test the artificial flower was fixed by means of a steel wire vertically at the inner wall of the experimental flight cage. For the preparation of the test stimuli, small circular disks with a diameter of 5.5 mm were cut out of filter paper (Rundfilter MN615; Macherey-Nagel, Düren, Germany) with a perforator and were treated with the test stimuli (Table 1). Between one and five stimuli were applied to the small disks as follows: 13.1 mg glass pellets (Worf-Glasskugeln Ø 40–80 μm) were transferred with forceps and fixed with glue (Methylan® by Henkel, Germany) onto the disk. This glue is pure methylcellulose and thus has no smell; the solvent control also contained methylcellulose and never attracted bumblebees. 10 μl quercetin powder suspended in distilled water (quercetin-dihydrate, 97%, Alfa Aesar®; normal concentration 3.357 × 10⁻⁵ mol/ml; high concentration 3.357 × 10⁻⁴ mol/ml) was transferred with a pipette onto the disk. The volume of 2 μl L-proline (Merck®, Darmstadt; 1.3897 × 10⁻⁴ mol/ml in destilled water), 10 μl glucose-monohydrate (Merck®, Darmstadt; 8.8810 × 10⁻⁴ mol/ml in destilled water), and 10 μl eugenol (Merck®, Darmstadt; 6.5164 × 10⁻⁵ mol/ml in n-hexane) were added using a pipette. After the application of one substance, the disk was dried for some minutes until the next substance was applied. Gustatory stimuli were applied last in order to avoid overlaying effects of other substances. To avoid transmission of odors or other contaminants, gloves were used while handling the artificial flower.

Dandelion pollen (Taraxacum officinale) was collected in the months from April to August 2014 and was fixed to the small filter paper disk with glue (Methylan® by Henkel, Germany). The plants were picked in the botanical garden of the Heinrich-Heine-University, Düsseldorf, Germany prior to pollen presentation. After they were kept for 1 day

| Substance                  | Supplier            | Effect     | Amount applied to disk                                      |
|----------------------------|---------------------|------------|-------------------------------------------------------------|
| Quercetin-dihydrate        | Alfa Aesar®         | Visual     | 10 μl of 3.6 × 10⁻⁵ mol/ml in distilled water               |
| Eugenol                    | NORMAPUR®          | Olfactory  | 10 μl of 6.5 × 10⁻⁵ mol/ml in hexane                        |
| Glucose-monohydrate        | Merck               | Gustatory  | 10 μl of 8.9 × 10⁻⁴ mol/ml in distilled water               |
| L-Proline                  | Merck               | Gustatory  | 2 μl of 1.4 × 10⁻⁴ mol/ml in distilled water                |
| Glass pellets              | Worf Glaskugeln     | Tactile    | 13.1 mg (40–80 μm), methylcellulose paste                   |
in containers with water, the fresh pollen was extracted, and preserved in the refrigerator. Dandelion pollen was chosen because the plants are known to have a long flowering period and thus fresh pollen was available throughout the experimental testing. Dandelion pollen is collected by bumblebees as a major protein source (Teper, 2006; Whittington, Winston, Tucker, & Parachnowitsch, 2004), although bumblebees cannot live on pure dandelion pollen diet (Génissel, Aupinel, Bressac, Tasei, & Chevrier, 2002). The diameter of dandelion pollen grains is about 25–30 μm.

All solvents used to prepare the stimulus combination were applied to the small filter paper disk of the solvent control.

Three test stimuli—stimulus combination, pollen, and solvent control—were fixed with glue (Methylan® by Henkel, Germany) to a circular filter paper (Rundfilter MN615; Ø 70 mm, Macherey-Nagel) in a distance of about 45 mm from each other (Figure 1). For each test series a minimum of twelve round filters were prepared each with three prepared disks, resp. test stimuli, and used as replicates. These three disks were fixed in all possible spatial arrangements to avoid position effects. All spatial arrangements were offered in equal numbers in the tests.

2.3 | Experimental procedure

The spontaneous behavior of single flower-naive bumblebees was tested using a single artificial flower displaying the triple choice test. The bumblebees were not trained before the test trial.

The experiments were performed between 8 a.m. and 4 p.m. Only one flower-naive individual at a time was released in the experimental flight cage and its behavior was recorded by a camera, Casio Exilim Ex-F1 equipped with a 36–432-mm objective. High-speed videos were recorded by this digital camera, positioned in an angle of 20° to the surface of the artificial flower and outside of the test cage, so that the activities of the bumblebees were clearly visible on the frames. To distinguish bumblebees that had already been tested from experimentally naive ones, the animals were tagged after the experimental trial with a numbered label (Ophalithplättchen, Holtermann, Brockel, Germany). All workers were naive and used only for one test.

The videos were used to categorize the behavioral reactions, which were defined as follows:

An approach is classified if the bumblebee approaches unequivocally one of the three test stimuli and targets at it by directing the flagellum of its antennae at the test stimulus distance and the antennal tips being closer than 1 cm. The distance between antennal tips and target stimulus was estimated by using the threefold length of the flagellum. An antennae reaction is classified if the bumblebee’s antennae are in physical contact with the artificial flower after an approach. A landing is classified if all 6 legs of the bumblebee are in physical contact with the artificial flower after an antennal reaction.

Proboscis extension, mandible biting, and buzzing were exhibited only rarely and in an irregular sequence.

The position of the test stimuli, uni-/multimodal stimulus combination, natural dandelion pollen, and solvent control were changed with each test to exclude the effect of position preferences of the bumblebees. A test series ended when twelve tested bumblebees showed at least one reaction. In three cases we tested 24 instead of 12 bumblebees.

With help of the video, the behavioral reactions of individual bumblebee workers were classified and indexed as follows.

\[
\text{Index of behavior (IB)} = \frac{\text{Number of reactions to stimulus}}{\text{Number of reactions to stimulus} + \text{Number of reactions to dandelion pollen}}
\]

Index of behavior (IB) is a parameter with values between 0 and 1. IB < 0.5 indicates that the frequency of bumblebees’ reactions to the dandelion pollen is higher than that to the tested stimulus combination. IB = 0.5 means bumblebees reacted to pollen and to stimulus combination equally often. IB > 0.5 indicates that the frequency of bumblebees’ reactions to the tested stimulus combination (with 1–5 stimuli) surpasses that of the dandelion pollen. Reactions toward the solvent control were never observed and thus are not included in the calculation of IB. The median value of IB calculated for all bumblebee workers (n = 12 or 24) that responded to the same stimulus was calculated. IB values for all bumblebee workers that responded to the same stimulus were tested against 0.5 (i.e., equal number of responses to stimulus and dandelion pollen) using a Wilcoxon signed-rank test. In addition, we corrected for multiple tests using the false discovery rate (Benjamini & Hochberg, 1995). Significant deviations from 0.5 indicate either preferences for dandelion pollen (IB < 0.5) or stimuli (> 0.5).
statistical analyses were performed using the statistical software R (R Core Team 2015).

3 | RESULTS

The tested bumblebees regularly exhibited approaches, antenna reactions and landings at one or two of the three close-range stimuli presented at a single disk (Figure 2). The bumblebees never responded to the solvent control and thus the solvent control was not used to calculate the IB. In total, 773 approaches of bumblebee workers toward the dandelion pollen were registered. 652 (84.3%) of these approaches ended up with an antenna reaction, and 517 (66.9%) with a landing reaction. Relatively few approaches led to proboscis extension (5.0%), mandible biting (6.0%), and/or buzzing (4.9%). The bumblebees approached the various stimulus combinations 183 times. 73.2% of these approaches ended up with antennal reaction, 56.8% with a landing reaction. In only 0.5% the bumblebees approached led to a proboscis extension, in 1.1% to mandible biting, and in 0.5% to buzzing (Figure 2).

The number of bumblebees that responded to dandelion pollen as constant stimulus in all tests was high and consistent: 11.04 ± 1.07 and a minimum of 9 out of 12 tested workers in one test series approached the dandelion pollen (Table S1); 10.82 ± 1.26 and a minimum of 8 workers antennated at it (Table S2), and 9.64 ± 1.92 and a minimum of 5 workers landed on it during the test interval of 1 min (Table S3). Proboscis extension, mandible biting, and buzzing were only rarely observed (Tables S4–S6).

In contrast, the bumblebees approached, antennated, and landed at the stimulus combination only if quercetin was present excluding some exceptions. In the 13 experiments in which the stimulus combination did not include quercetin, the bumblebees approached the stimulus combination in 1.2% of all responses, whereas 98.8% of the approaches were directed to the dandelion pollen. In those 15 experiments, in which the stimulus combination included quercetin, the bumblebees approached the stimulus combination in 31.0% of all responses, and 69.0% of the approaches were directed to the dandelion pollen (Figure 3). When quercetin was present in the stimulus combination 7.47 ± 1.31 and a minimum of five individuals approached the stimulus combination (Table S1), 6.13 ± 1.93 and a minimum of two bumblebees antennated at it (Table S2), 5.07 ± 1.84 and a minimum of one bumblebee landed on it within the experimental test interval (Table S3).

For the most complete stimulus combination, including quercetin, eugenol, glucose, proline, and glass, the number of bumblebees responding at least once was as high as for dandelion pollen with regard to approach, antennal reaction, and landing, and further reactions such as buzzing, proboscis extension, and mandible biting (Tables S4–S6).

The bumblebees’ further responses were evaluated for those tests, in which the stimulus combination included the visual stimulus quercetin: The portion of approaches that were followed by an antennal reaction was not significantly different for approaches toward dandelion pollen (84.3%) and approaches toward the stimulus combinations including quercetin (73.2%; Fisher’s exact test, \( p = .295 \); Figure 4).
Moreover, the portion of antennal reactions that ended up with a landing was also not significantly different for antennal reactions at dandelion pollen (79.3%) and antennal reactions at the stimulus combinations including quercetin (77.6%; Fisher’s Exact test, \( p = .940 \); Figure 5). However, significantly more landings on dandelion pollen as compared to landings on the stimulus combinations including quercetin were followed by mandible biting, proboscis extension or buzzing (24.8%, resp. 3.8% (Fisher’s exact test, \( p < .001 \)).

No general correlation was found for the portion of antennal reactions that ended up with a landing and the number of tactile or chemical stimuli presented at stimulus combinations including quercetin (\( r = -.013; p = .965 \); Pearson). However, the number of stimuli combined in a stimulus combination seemingly predicts the frequency of responses of the bumblebees toward the stimulus combination. In only 4 experiments (17: Que Eug Pro; 22: Que Eug Gla; 26: Que Eug Glu Pro; 28: Que Eug Glu Pro Gla) did the bumblebees antennate more often at the test stimulus combination (containing quercetin) following an approach when compared to dandelion pollen; all these four stimulus combinations contained eugenol (Figure 4, Tables S2 and S7). Moreover, these were the only stimulus combinations for which the portion of antennal reactions that ended up with a landing was larger for the stimulus combination than for the dandelion pollen (Figure 5, Table S7).

**DISCUSSION**

Our experimental results demonstrate that the common pollen pigment quercetin is necessary and sufficient to elicit a spontaneous full behavioral response in previously inexperienced bumblebees including approach, antennal reaction and landing, confirming that visual signals are highly important to trigger innate responses to pollen signals (Lunau, 1992, 2000). In some experiments the bumblebees responded equally or even better to the stimulus combination than to the natural dandelion pollen indicating attractive stimuli in the stimulus combination. Additional stimuli thus might facilitate pollen detection and increase the frequency of subsequent behavioral reactions such as antennal contact and landing, whereas the bumblebees did not respond to olfactory stimuli alone, emphasizing the efficacy of multimodal stimuli for pollen detection and recognition. These results indicate that multimodal signals are not only important for flower recognition (Junker & Parachnowitsch, 2015), but also for pollen recognition in naïve bumblebees. The importance of interactions between floral traits of different sensory modalities for various flower visitors...
including bees have been emphasized by Junker and Parachnowitsch (2015) and Rüdenauer et al. (2015). For experienced bees, it has been demonstrated that pollen odor strongly affects the bees’ foraging behavior (Cook et al., 2005).

The results of this study strengthen the view that multimodal stimuli are responsible for triggering full behavioral responses to pollen in bumblebees, which is particularly evident in behavioral reactions following the visually guided approach. For the decision to land on a flower, the visual stimulus alone is sufficient, but additional stimuli of other modalities increase the frequency of landings. Of note, a higher concentration of the pigment quercetin also resulted in an increase in approaches suggesting that the salience of the visual stimulus (compare to Katzenberger et al., 2013) in the normal stimulus combinations was suboptimal; however, the difference in the frequency of approaches toward the normal and the tenfold concentration of quercetin was not significant. UV-absorbing yellow is by the far the most common pollen color in plants and caused by flavonoid pigments known for antifungal and antibacterial properties and shielding against the mutagenic UV light (Lunau, 1995, 2000; Osche, 1979; Pacini & Hesse, 2005). However, preference tests with bumblebees suggest that the UV-absorbing yellow color hue is not the decisive color parameter triggering the bumblebees’ antennal response (Lunau, Wacht, & Chittka, 1996; Rohde, Papirek, & Lunau, 2013). There is, however, evidence that visual pollen cues play a dominant role in pollen detection by bees. Bumblebees visiting natural flowers exhibit the antennal reaction not only at stamens, but also at stamen-mimicking floral guides (Lunau, 1991, 1992). Stamen- and pollen-mimicking floral guides are a very common feature of bee-pollinated flowers and mostly display a UV-absorbing yellow color (Lunau, 2000, 2006) that is contrasting against the rest of the flower (Lunau, 1992). Conspicuous pollen- and stamen-mimicking floral guides are more likely to attract bees for antennal contact if combined with less attractive, that is, not UV-absorbing and yellow, real pollen and stamens (Lunau, 2006).

In our experiments, chemical and tactile stimulants of the stimulus combinations slightly increase the frequency of decision for closer inspection of the stimulus combinations. It is known that pollenkitt is a solvent for chemical pollen stimuli, but neither a common pollen odor (Dobson, 1988; Dobson & Bergström, 2000) nor a typical pollen taste has been described (Pacini & Hesse, 2005). An exception is the free amino acid proline which is a common constituent of the pollenkitt of angiosperm pollen grains (Lehmann et al., 2010; Stanley & Linskens, 1974; Weiner, Hilpert, Werner, Linsenmair, & Blüthgen, 2010) and thought to support pollen germination and pollen tube growth due to its hygroscopic property (Britikov & Musatova, 1964; Dashek, 1970). Due to its widespread presence in pollenkitt, proline would represent a suitable key stimulus for pollen detection. Electrophysiological studies with the labellar and tarsal taste bristles of the pollen-feeding syrphid fly Eristalis tenax have shown that the salt receptor in these flies is much more sensitive to the amino acid proline than to cations and...
might be regarded as a proline receptor (Wacht, Lunau, & Hansen, 2000). It is unlikely that proline is detected prior to contact due to its poor olfactory detectability (Linander, de Ibarra, & Laska, 2012).

There is little experimental evidence that tactile cues might be important for triggering distinct behavioral responses in pollen-collecting insects. Bees are known to collect chemically inert pollen surrogates such as glass powder or cellulose powder from emasculated or artificial flowers (Burkart, Schlindwein, & Lunau, 2014; Konzmann & Lunau, 2014).

Relatively few bumblebees tried to harvest the pollen by extending the proboscis, biting with the mandibles or buzzing. They exhibited these behavioral reactions only rarely, but significantly more often at the natural dandelion pollen as compared to the stimulus combination. Only the stimulus combinations including all tested stimuli, namely quercetin, eugenol, glucose, proline, and glass powder, elicited as many responses as the natural pollen; this was valid for all reactions such as approach, antennation, landing, and subsequent reactions. This result may be interpreted that additive effects of various stimuli contributing to the attractiveness of pollen and stimulus combination. Lunau et al. (2015) found conflicting evidence for key stimuli eliciting pollen collection in bumblebees, which readily collected chemically inert pure glass powder, stopped collecting pollen if it was embittered by quinine obviously only after probing pollen eventually. Some flowers hide their pollen grains poricidal anthers and bees buzz these flowers even if the anther pores have been glued indicating that they are obviously unable to detect visual or chemical pollen signals before buzzing the flowers, but immediately recognize pollen release (Burkart et al., 2014).

Testing bumblebees which were naive in regard to pollen would have improved this study. However, the bumblebees’ experience with natural pollen cannot be excluded, because all bumblebees feed on pollen as larvae and experience pollen odor in the nest. By feeding pollen in the dark nest, the premature conditioning of the yellow pollen color could be avoided.

The presented data suggest that multimodal pollen stimuli are effectively stimulating pollen collection behavior in bumblebees and that a visual color stimulus seems essential to start the behavioral sequence. The hierarchy of multimodal cues is seemingly linked to the sequence of behavioral reactions, initiated by the visually guided approach and continued by olfactory, gustatory, and tactile stimuli triggering subsequent reactions. Multimodal stimulus combinations were able to trigger bumblebees’ responses as strong as natural dandelion pollen. Although the chemical stimuli tested in this study might be rather common among pollen, none of eugenol, proline, or sucrose is known as key substance for bees to identify pollen (Dobson & Bergström, 2000). It remains open, how a somehow arbitrary, but multimodal combination of a small number of stimuli, can trigger responses as strong as natural pollen, but it seems likely that addressing several sensory modalities, that is, a multimodal stimulus, is important to trigger pollen seeking behavior.
### REFERENCES

Ägren, L., & Hallberg, E. (1996). Flagellar sensilla of bumble bee males Hymenoptera, Apidae, Bombus. Apidologie, 27, 433–444.

Alford, D. V. (1975). Bumblebees. London: Davis-Poynter Ltd.

Arenas, A., & Farina, W. M. (2012). Learned olfactory cues affect pollen-foraging preferences in honeybees. Animal Behaviour, 83, 1023–1033.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B, 57, 289–300.

Burdon, R. C., Raguso, R. A., Kessler, A., & Parachnowitsch, A. L. (2015). Ontogenetic constraints from floral development, colour pattern rules and scents: Insights from bees and flowers. Journal of the Royal Society B: Biological Sciences, 282, 153–166.

Cook, S. M., Sandoz, J.-C., Martin, A. P., Murray, D. A., Poppy, G. M., & Carter, C., Shafir, S., Vaknin, L., Palmer, R. G., & Thornburg, R. (2006). A network. Evolution of Comparative Physiology A, 39, 123–137.

Dobson, H. E. M., Danielson, E. M., & Van Wesep, I. D. (1999). Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). Plant Species Biology, 14, 153–166.

Dötterl, S., & Jürgens, A. (2005). Spatial fragrance patterns in flowers of *Silene latifolia*: Lilac compounds as olfactory nectar guides? Plant Systematics and Evolution, 255, 99–109.

Endress, P. K., & Steiner-Gafner, B. (1996). Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge University Press.

Frost, E. H., Shutler, D., & Hillier, N. K. (2012). The proboscis extension reflex to evaluate learning and memory in honeybees (Apis mellifera): Some caveats. Naturwissenschaften, 99, 677–686.

Génissel, A., Aupinel, P., Bressac, C., Tasei, J.-N., & Chevrier, C. (2002). Influence of pollen origin on performance of *Bombus terrestris* microcolonies. Entomologia Experimentalis et Applicata, 104, 329–336.

Gil, M., Menzel, R., & De Marco, R. (2008). Does an Insect’s unconditioned response to sucrose reveal expectations of reward? PLoS ONE, 3, e2810.

Giurfa, M., Hammer, M., Stach, S., Stollhoff, N., Müller-Deisig, N., & Mizrycki, C. (1999). Pattern learning by honeybees: Conditioning procedure and recognition strategy. Animal Behaviour, 57, 315–324.

Goulson, D. (2003). Bumblebees – Their behaviour and ecology. Oxford: Oxford University Press.

Grüter, C., Arenas, A., & Farina, W. M. (2008). Does pollen function as a reward for honeybees in associative learning? Insectes Sociaux, 55, 425–427.

Harder, L. D., & Thomson, J. D. (1989). Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. American Naturalist, 133, 323–344.

Haupt, S. S. (2004). Antennal sucrose perception in the honey bee (Apis mellifera L): Behaviour and electrophysiology. Journal of Comparative Physiology A, 190, 735–745.

Howell, A. D., & Alarcón, R. (2007). Osmia bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. Animal Behaviour, 74, 199–205.

Junker, R. R., Blüthgen, N., Brehm, T., Binkenstein, J., Paulus, J., Schaefer, H. M., & Stang, M. (2013). Specialisation on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. Functional Ecology, 27, 329–341.

Junker, R. R., & Parachnowitsch, A. L. (2015). Working towards a holistic view on flower traits – How floral scents mediate plant-animal interactions in concert with other floral characters. Journal of the Indian Institute of Science, 95, 43–67.

Katzenberger, T. D., Lunau, K., & Junker, R. R. (2013). Salience of multi-modal flower cues manipulates initial responses and facilitates learning performance of bumblebees. Behavioural Ecology and Sociobiology, 67, 1587–1599.

Kevan, P. G., & Lane, M. A. (1985). Flower petal microtexture is a tactile cue for bees. Proceedings of the National Academy of Science USA, 82, 4750–4752.

Konzmann, S., & Lunau, K. (2014). Divergent rules for pollen and nectar foraging bumblebees – A laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. PLoS ONE, 9, e91900.

Lehmann, S., Funk, D., Szabados, L., & Rentsch, D. (2010). Proline metabolism and transport in plant development. Amino Acids, 39, 949–962.

Lehner, M., Horridge, G. A., Zhang, S. W., & Gadagkar, R. (1995). Shape vision in bees: Innate preference for flower-like patterns. Philosophical Transactions of the Royal Society B: Biological Sciences, 347, 123–137.

Leonard, A. S., & Masek, P. (2014). Multisensory integration of colors and scents: Insights from bees and flowers. Journal of Comparative Physiolog A, 200, 463–474.

Linander, N., de Ibarra, N. H., & Laska, M. (2012). Olfactory detectability of L-amino acids in the European honeybee (Apis mellifera). Chemical Senses, 37, 631–638.
Lipp, J. (1991). Detection of ABA and proline in pollen. *Biochemie und Physiologie der Pflanzen*, 187, 211–216.

Lunau, K. (1991). Innate flower recognition in bumblebees (*Bombus terrestris*, B. lucorum; Apidae); Optical signals from stamens as landing reaction releasers. *Ethology*, 88, 203–214.

Lunau, K. (1992). Innate recognition of flowers by bumblebees; Orientation of antennae to visual stamen signals. *Canadian Journal of Zoology*, 70, 2139–2144.

Lunau, K. (1995). Notes on the colour of pollen. *Plant Systematics and Evolution*, 198, 235–252.

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

Lunau, K. (2006). Stamens and mimic stamens as components of floral colour patterns. *Botanische Jahrbücher für Systematik, Pflanzengeographie und Pflanzenzüchtung*, 127, 13–41.

Lunau, K. (2011). Warnen, Tarnen, Täuschen. Mimikry und Nachahmung bei Pflanze, Tier und Mensch. Darmstadt: Wissenschaftliche Buchgesellschaft.

Lunau, K., Fieselmann, G., Heuschen, B., & van de Loo, A. (2006). Visual targeting of components of floral colour patterns in flower-naïve bumblebees (*Bombus terrestris*; Apidae). *Naturwissenschaften*, 93, 325–328.

Lunau, K., Piorek, V., Krohn, O., & Pacini, E. (2015). Just spines-Mechanical defence of malicious pollen against collection by corbiculate bees. *Apidologie*, 46, 144–149.

Lunau, K., Wacht, S., & Chittka, L. (1996). Colour choices of naïve bumble bees and their implications for colour perception. *Journal of Comparative Physiology A*, 178, 477–489.

Menzel, R. (1993). Associative learning in honey bees. *Apidologie*, 24, 157–157.

Nepi, M., Guarnieri, M., & Pacini, E. (2003). “Real” and feed Pollen of *Lagerstroemia indica*: Ecophysiological differences. *Plant Biology*, 5, 311–314.

Osche, G. (1979). Zur Evolution optischer Signale bei Blütenpflanzen. *Biologie in unserer Zeit*, 9, 161–170.

Osche, G. (1983). Optische Signale in der Coevolution von Pflanze und Tier. *Berichte der Deutschen Botanischen Gesellschaft*, 96, 1–27.

Pacini, E., & Hesse, M. (2005). Pollenkitt - its composition, forms and functions. *Flora*, 200, 399–415.

Pohl, M., & Lunau, K. (2007). Modification of the innate antennal reaction at floral guides in experienced bumblebees, *Bombus terrestris* (Hymenoptera: Apidae). *Entomologica Generalis*, 29, 111–123.

R Core Team (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Raguso, R. A. (2004). Flowers as sensory billboards: Progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology*, 7, 434–440.

Raguso, R. A. (2009). Floral scent in a whole-plant context: Moving beyond pollinator attraction. *Functional Ecology*, 23, 837–840.

Rohde, K., Papiorek, S., & Lunau, K. (2013). Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. *Journal of Comparative Physiology A*, 199, 197–210.

Rouliston, T. H., & Cane, J. H. (2000). Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, 222, 187–209.

Rüdenauer, F. A., Späthe, J., & Leonhardt, S. D. (2015). How to know which food is good for you: Bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *Journal of Experimental Biology*, 218, 2233–2240.

Schmidt, J. O., & Hanna, A. (2006). Chemical nature of phagostimulants in pollen attractive to honeybees. *Journal of Insect Behavior*, 19, 521–532.

Stanley, R. G., & Linskens, H. F. (1974). *Pollen: Biology, biochemistry, management*. Berlin: Springer-Verlag.

Stanley, R. G., & Linskens, H. F. (1985). *Pollen: Biology, biochemistry, Urs Freund Verlag, Gewinnung and Verwendung; Greifenberg/Ammerssee.*

Teper, D. (2006). Fodder plants of *Bombus terrestris* L. as determined by pollen analysis of faeces. *Journal of Apicultural Research*, 50, 101–108.

Wacht, S., Lunau, K., & Hansen, K. (1994). Optical and chemical stimuli control pollen feeding in the hoverfly *Eristalis tenax* L. (Syrphidae, Diptera). *Entomologia Experimentalis et Applicata*, 80, 50–53.

Wacht, S., Lunau, K., & Hansen, K. (2000). Chemosensory control of pollen ingestion in the hoverfly *Eristalis tenax* L. by labellar taste hairs. *Journal of Comparative Physiology A*, 186, 193–203.

Weiner, C. N., Hilpert, A., Werner, M., Linsenmair, K. E., & Blüthgen, N. (2010). Pollen amino acids and flower specialisation in solitary bees. *Apidologie*, 41, 476–487.

Whittington, R., Winston, M. L., Tucker, C., & Parachnowitsch, A. L. (2004). Plant-species identity of pollen collected by bumblebees placed in greenhouses for tomato pollination. *Canadian Journal of Plant Science*, 84, 599–602.

Willmer, P. (2011). *Pollination and floral ecology*. Princeton: Princeton University Press.

Wykes, G. R. (1952). An investigation of the sugars present in the nectar of flowers of various species. *New Phytologist*, 51, 210–215.

Zhang, F. P., Larson-Rabin, Z., Li, D. Z., & Wang, H. (2012). Colored nectar as an honest signal in plant-animal interactions. *Plant Signal & Behaviour*, 7, 811–812.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Wilmsen S, Gottlieb R, Junker RR, and Lunau K. Bumblebees require visual pollen stimuli to initiate and multimodal stimuli to complete a full behavioral sequence in close-range flower orientation. *Ecol Evol*. 2017;7:1384–1393. https://doi.org/10.1002/ece3.2768