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REVIEWS

# Working Towards a Holistic View on Flower Traits—How Floral Scents Mediate Plant–Animal Interactions in Concert with Other Floral Characters

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**Abstract |** Flowers are complex structures, synchronously displaying both olfactory and visual signals/cues in the context of a particular floral morphology, that also vary in resource quantity and quality. Despite or possibly because of this complexity, many studies focus on a single or few traits rather than studying floral phenotypes in a more integrated fashion. However, each of these distinct trait classes (signals/cues, morphology and resources) mediates interactions with floral visitors, demanding a more holistic view of flowers. In our review, we integrate floral scents into the broader context of the whole-flower phenotype. We discuss the functions of scent bouquets, colouration, morphology and rewards in flower–visitor interactions from an ecological and evolutionary perspective in isolation and taken together. Studies demonstrate that floral scent bouquets can act additively or synergistically with other modalities, and that their effects on flower visitors are context-dependent. We also present field study results showing that reward levels modulate dose-dependent responses to volatiles by honeybees. To motivate studies examining complex floral phenotypes, we outline statistical approaches suited to deal with the complex multivariate datasets generated by these studies. We conclude with a discussion on why flowers display multimodal traits and suggest future research efforts. Our aim is to foster a fresh view on integrated floral phenotypes and stimulate studies exploring the combined effects of olfactory, visual, morphological and nutritional traits on flower–animal interactions.

**Keywords:** *antagonists, behaviour, morphology, mutualism, pollination, rewards, volatile organic compounds*

## 1 Introduction

Angiosperms are the most diverse group of vascular plants, in part due to sexual reproduction mediated by pollen-transfering animals. The major advantage of biotic pollination is directed transmission of pollen between anthers and stigmas of conspecific plants. This requires either specialist animals that only visit flowers of one or a limited spectrum of plant species, or generalist animals that acquire short-term specialisation on species known as flower fidelity or constancy.[1] Both innately specialised animals and those specialized *via* associative learning are dependent on floral cues/signals to distinguish among plant species. Likewise, plants are heavily dependent on the same cues/signals to facilitate cross-pollination and to avoid wasting pollen or receiving pollen from heterospecifics. Floral volatiles are key characters in mediating flower–visitor interactions, attracting generalist and specialist pollinators, structuring flower–visitor communities and coping with antagonists.[2–5] Acknowledging the

**Constancy:** is the tendency for pollinators to visit one flower type in foraging bouts despite the presence of other rewarding flowers in a community.

**Associative learning:** describes the ability of flower visitors to associate floral traits with rewards leading to acquired (short term) preferences for these traits.

**Pollinators:** are animals such as insects, birds and mammals that transfer pollen from anthers of one flower to stigmas of another.

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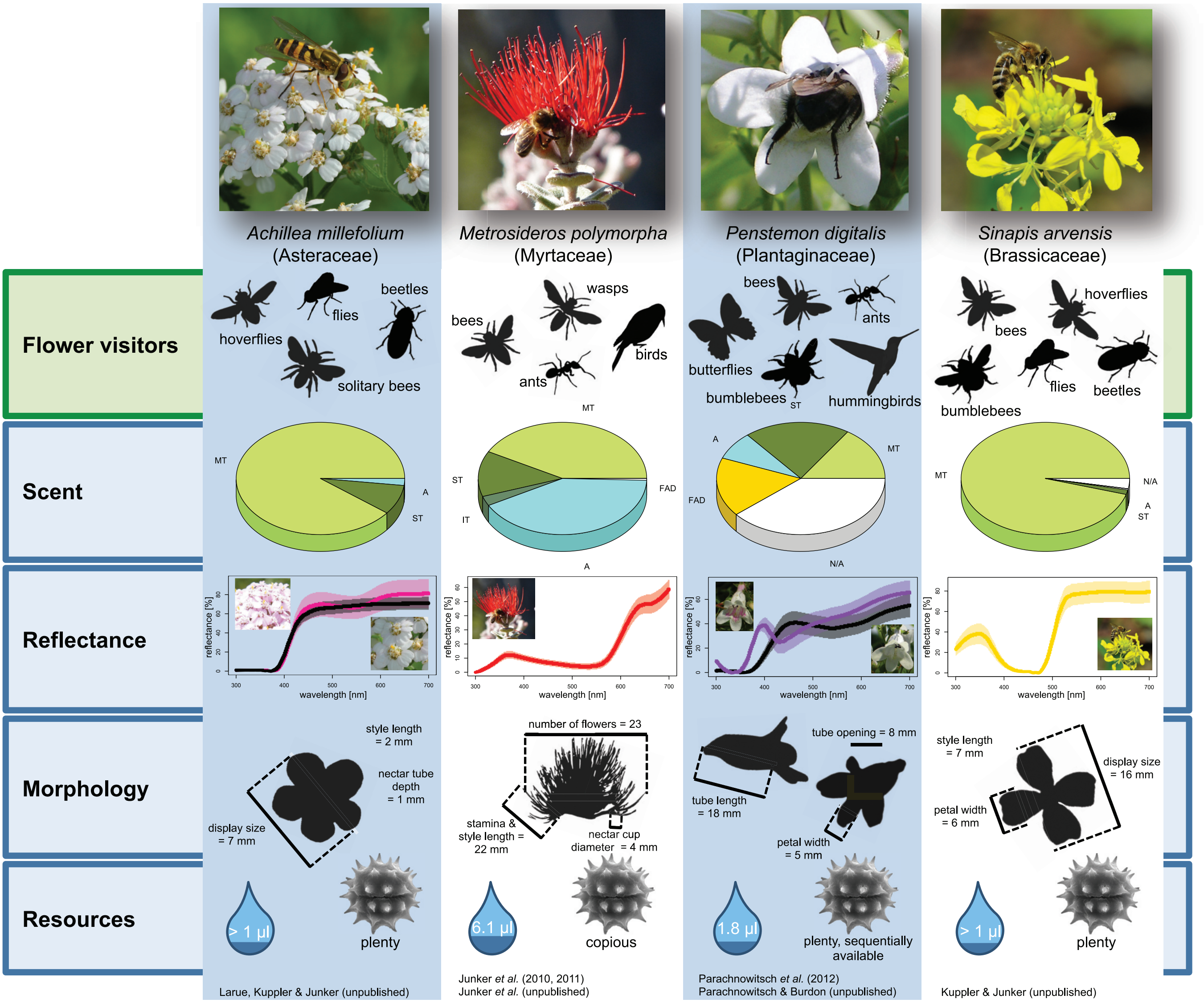
importance of this flower trait, the composition, biosynthesis, ecological functions, evolution and modes of action of scent bouquets have been comprehensively summarized.[6–10]

In plant–animal communication, information is rarely transmitted by a single sensory modality; the partners usually rely on multiple modalities. Important goals in multimodal communication research are to quantify the relative importance of each modality and the additive or synergistic effects emerging from multimodal signalling.[11,12] These questions are particularly interesting in flower–visitor interactions where plant reproductive structures display several cues or signals perceivable by the olfactory, visual, tactile and gustatory sensory abilities of the flower visitors. In fact, flower visitors can respond to volatiles, colours, shapes and patterns prior to landing on flowers, following which they are confronted with the flower's architectural properties, and can evaluate the quality and quantity of flower rewards (Fig. 1). In mechanistic and behavioural studies addressing questions about the cognitive abilities of flower visitors, as well as in studies on the evolution of flower traits or ecological processes, often only one (or a few) of these flower traits is fully considered. Such studies can provide detailed insights but neglect potential interaction effects with other traits.

This trend towards unimodal investigations is surprising since a holistic view on flower traits has a long tradition in pollination biology where suites of (multimodal) traits are distilled down to syndromes. Pollination syndromes are used to describe the general features of floral phenotypes pollinated by the same functional groups[13,14] and assign colours, flower shape, size and orientation, the presence or absence of nectar guides, rewards and key elements of scent characteristics (see[15] for chemically informed assignment) to flowers. These functional groups include, for example, melittophilous, myophilous or ornithophilous flowers that are pollinated by bees, flies or birds, respectively.[16,17] The simplification of the floral characters (usually nominal scaled descriptions) used in classical syndromes makes this concept very appealing for field investigations and teaching but makes it also susceptible to misinterpretations and flawed predictions. For example, according to common descriptions of the syndromes, a small, white, bell shaped, weakly scented, horizontally oriented and radially symmetrical flower with exposed sexual organs, offering nectar and/or pollen[17] and emitting benzenoids, fatty acid derivates, terpenoids and/or nitrogen-containing compounds[15] can either be classified as melittophilous or myophilous.[15,17] Examples like this may explain why the phenotype of a large proportion of flowers fails to predict the most common pollinator.[16] However, other studies succeed in predicting the main pollinator in a larger proportion of flowers.[17] Furthermore, evidence has accumulated that flower traits are not only selected by pollinating mutualists but also by antagonistic agents such as florivores that reduce the reproductive success of plants when visiting flowers, e.g.[18–20] Additionally, animal taxa choose flowers based on species-specific sets of traits, i.e. not all traits are important for each animal taxon.[21] These factors may interfere with predictions on the main pollinator based on suites of flower traits. Nonetheless, the underlying concept of pollination syndromes, i.e. that plants sharing the "most effective pollinator" often also share several flower traits,[22] is undisputed and has been shown in studies that quantitatively measured morphological or chemical flower traits, e.g.[23,24]

**Pollination syndromes:** are suites of floral traits that are shared by flowers pollinated by the same functional group such as bees, beetles, birds or bats. Traits include colours, flower shape, size and orientation, the presence/absence of nectar guides, rewards and sometimes scent characteristics of flowers, and have been used for a first estimation of the most common pollinators. However, note that syndromes are based on simplifications and that the generality of the concept is controversial.

The goal of this review article is to re-adopt the approach of pollination syndromes by integrating measurements on visual, morphological and nutritional traits (in a ratio rather than nominal scale) into detailed investigation of floral scent-mediated flower–animal interactions. Therefore, we first lay the basis for the understanding of trait-mediated interactions by summarising the ecological functions and evolution of flower scents and by evaluating whether flower scents act as a bouquet or whether individual volatiles have distinct functions. This introduction into floral volatiles and their effects on animal visitors is complemented by brief summaries on visual, morphological and nutritional traits. The interaction effects between volatiles and the other traits are discussed to demonstrate that floral scent bouquets and individual volatiles act in additive or synergistic ways with other modalities, and that their effects on flower visitors are context-dependent and can be modulated by the whole-flower phenotype. The literature review is supplemented by a case study showing that dose-dependent responses to volatiles by honeybees vary with the reward level offered by artificial flowers. Finally, we briefly suggest and discuss statistical approaches suited to analyse the complex multivariate datasets obtained in studies quantifying multiple traits. We conclude with a discussion on why flowers display traits of several modalities in the light of the ecology and evolution of flower–visitor interactions. Finally we suggest future perspectives that will foster a fresh view on the integrated floral phenotype including olfactory, visual, morphological and nutritional traits.



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Kuppler & Junker (unpublished)

***Figure 1:*** Holistic view on flower traits, including olfactory, visual, morphological and resource-based traits, as well as the main flower visitors of four plant species selected from our own research is shown. Scent samples were analysed using gas chromatography coupled with a mass spectrometer (MT = monoterpenes, ST = sesquiterpenes, IT = irregular terpenes, A = aromatics, FAD = fatty acid derivates, N/A are unidentified substances). Floral reflectance was measured with a photospectrometer equipped with a standardised light source (300–700 nm). Floral morphology was measured using a calliper rule. Nectar was extracted using micro-capillaries, concentration was measured with a refractometer with the exception of *P. digitalis* where concentration was estimated in the lab and is indicated by the level of the nectar droplet. Information on pollen amounts are estimates based on our observations. Flower visitors may be mutualists, commensalists or antagonists. For a simplified representation of the traits, we depicted chemical scent classes, chose a restricted selection of morphological and nutritional characters and give mean values of the traits, only. Note that each of the traits is more complex than shown here and that intra-specific variability of the traits can be pronounced.

## 2 Floral Volatile Organic Compounds

Floral scents are comprised of volatile compounds specifically emitted by flowers and are what we perceive when we smell the pleasantness of a rose or the stink of a skunk flower. The list of volatiles grows with the ever increasing characterisation of floral scents.[10] A comprehensive review of the composition of floral volatiles is beyond the scope of this paper and can be found elsewhere[10,25] but generally, most floral volatiles fall into just a few compound classes, all of which have low molecular weight. The two largest groups of floral volatiles are the mono- and sesquiterpenoids and aliphatics, followed by aromatic compounds (benzenoids and phenylpropanoids) and sulphur and nitrogen containing compounds.[25] The four pathways of secondary metabolism that contribute most flower-specific volatiles are the pyruvate,

**Gas chromatography and mass spectrometry:** is used to separate individual compounds that constitute floral scent bouquets by their volatility, size and polarity using a temperature gradient and a chromatographic column. Mass spectrometry is used to reveal the characteristic ratio of fragments of compounds with specific weights after breakup due to bombardment with electrons. The mass spectrum in combination with a compound's retention time (time when leaving the column) allows identification of substances.

**Floral scents:** low molecular weight volatile organic compounds specifically emitted by flowers. Floral scent bouquets consist of few to many individual compounds deriving from limited biochemical pathways of secondary metabolism. Their ecological functions range from attraction of pollinators to defence against antagonistic flower visitors with consequences for the structuring of flower–visitor communities.

acetyl-CoA, lipoxygenase and shikimate/phenylalanine pathways, which synthesize mono- and sesquiterpenes, aliphatics, and aromatic compounds respectively.[9,10] Many compounds are shared among species across angiosperm phylogeny.[10]

Floral scents have often been viewed in terms of attraction and there is strong evidence from both plants and animals that mutualists use floral scents to locate floral resources. From the plant's perspective, scents should be produced when they will provide the most benefit. Flowers often increase emissions during the times when their pollinators are most active[26–28] and reduce[29–31] or alter[32] emission with successful pollination, indicating the importance of scent in pollination. A recent example also demonstrates the dramatic loss of floral scent with a shift in mating system from self-incompatibility (outcrossing) to self-compatibility (likely high selfing rates without pollinator visitation) in populations of *Abronia umbellata*.[33] From the pollinator's perspective, scents are useful if they provide reliable information about floral resources, and tests of pollinator behaviour (mostly in the lab) show that they can accurately learn rewarding flowers.[34–36] However, few studies directly examine how accurately floral scents convey resource status in natural settings. For example, Galen *et al.*[37] found no link between scent and nectar amounts at the flower level suggesting that plants may not be honest in their scent signalling. However, community context likely affects the scale at which floral honesty is important. For example, if a highly rewarding plant emits a unique scent or scent blend compared to less rewarding co-flowering plants, scent might still be an important cue guiding to nectar and pollen resources. However, more empirical and theoretical work is needed in understanding the importance of honesty in natural settings.[7,38,39]

The evolution of floral scents is likely complex but since pollination is the primary function of flowers, viewing floral traits through that lens is a good start.[35] For scents, some general trends can be seen between compounds emitted and the main pollinators of the species. Perhaps not surprisingly, some of the strongest evidence for convergence of floral scents comes from specialized plants that mimic insect oviposition sites where necrophagous, coprophagous or saprophagous insects would oviposit because oviposition sites such as dead animals and feces emit just a few common sulphur-containing compounds.[40] Similarly various studies[15] have sought and found characteristic volatiles attracting specific functional groups of pollinators such as bats,[41,42] moths,[43,44] butterflies,[45] or beetles.[46] Whereas many pollinator taxa are associated with particular compounds/compound classes, bird-pollinated species often lack floral scents or emit them in very low amounts.[47] However, some of the more generalist systems including bee pollination show vast diversity rather than well-defined patterns in scent emission,[15] including weakly scented plants. Consequently, much like other floral traits, the presence of a floral volatile should not be used as evidence for the type of pollinators of a species, without direct observation.

Floral scent function is clearly more than attraction.[48] Flowers are often visited by a variety of antagonists that can also drive natural selection on floral traits.[20,49,50] Thus, floral volatiles are likely shaped by interactions with both mutualists and antagonists.[4,48] Support for selection by antagonists on floral volatiles comes from the repellent effect of floral volatiles in a number of systems.[37,51,52] Furthermore, the set of insect-produced and floral volatiles have a large overlap and 'floral' volatiles are often independently produced by flower visiting insects suggesting that plants may have co-opted volatiles used in insect communication for floral signalling.[53] Although researchers are beginning to examine selection on floral scent,[54–56] to date there are no direct experimental tests to definitively determine the relative importance of mutualists and antagonists in natural selection on floral volatiles.[57] However, evidence is mounting that floral volatiles likely evolved to fill a number of roles in plant–animal communication ranging from attraction to repellence.[4,6]

Floral scents are complex traits and floral volatile blends rarely contain only one or a handful of compounds but more often comprise several tens to sometimes over a hundred compounds. The chemical diversity of floral scents has lead to questions of how visitors perceive and respond to these complex bouquets. Researchers have explored two main hypotheses: either flower–visitor interactions are mediated by key substances (single volatiles) or several compounds in specific ratios (whole bouquet or a subset). Support for unique key substances is rare in host finding for herbivores, while specific ratios of common compounds find more support.[58] However, floral volatiles clearly have different roles compared to vegetative scents, the foremost difference being that plants actively attract their mutualists, while the same is not true for herbivores.

Evidence for key components of the floral bouquet mediating plant–insect interactions comes from a number of interactions including attraction of specialist and generalist mutualists, deception, and repellence of antagonists. For example, the single attractant of the specialist fig pollinator *Ceratosolen gravelyi*, 4-methylanisole, dominates the scent bouquet of its mutualistic partner *Ficus semicordata*.[59] Another specialist, the *Hadena bicruris* moth, relies on a single compound or few volatiles (lilac aldehydes) abundantly emitted by its host species *Silene latifolia*.[60] For *Datura wrightii* and the moth *Manduca sexta* a broader but specific subset of nine of 60 floral volatiles is required to evoke foraging.[61] This mix was as attractive as the whole *D. wrightii* floral scent and responses were comparable across a broad range of volatile concentrations. Interestingly, other plants from diverse families that also rely on *M. sexta* pollination have converged to produce at least some of the same key compounds[62] suggesting their importance for attraction. However, evidence for important key substances are not only evident from tightly co-evolved systems. For instance, generalist bees and moths are attracted to 1,4-dimethoxybenzene or lilac aldehyde respectively, both emitted by *Salix caprea*.[26] Key substances also mediate deceptive plant–pollinator interactions. The sexually deceptive orchid *Ophrys speculum* attracts pollinating *Campsoscolia ciliata* male wasps by the emission of oxygenated acids (mostly 9-hydroxydecanoic acid) in the same enantiomeric composition as the female sex pheromone. While the rest of the floral bouquet differs from that of female wasps, it does not impair male attraction showing that one (or few) key compounds are sufficient for mimicry.[63] For *Nicotiana attenuata* both key attractive and repellent substances in the floral scent bouquet mediate interactions with mutualists and antagonists. Benzyl acetone attracts hawkmoth and hummingbird pollinators, whereas nicotine decreases damage by florivores and nectar robbers.[52] Furthermore, nectar-thieving ants can be repelled by linalool[51,64] and 2-phenylethanol.[37]

Studies examining the learning abilities of insects have shown that insects use key substances within complex bouquets to associate rewards with volatiles and to discriminate between different bouquets, e.g.[65,66] For example, *Helicoverpa armigera* moths allowed to freely visit flowers, positively responded to only some volatiles of the natural scent bouquet, while the others remained behaviourally insignificant.[67] Likewise, when honeybees were confronted with individual substances that comprised an artificial mixture of volatiles to which they had been previously trained, some key substances elicited a high proportion of proboscis extensions (similar to the proportion of extensions when tested with the mix) but other substances evoked significantly lower proportions of responses.[68] Key substances are often most effectively learned individually,[69] inform most reliably about the reward[70] or are the most salient compounds[71,72] compared to the other compounds in a bouquet.

Unlike in host finding by herbivores,[58] there is much less evidence for the importance of specific ratios between components of the floral scent blend. However, in cases where sympatric figs share components of their floral scent bouquets, species-specific ratios of these substances result in a unique bouquet that evokes the attraction of their associated pollinating wasps.[73] Further, *Breynia vitis-idaea* emits a combination of two common floral volatiles to attract their specialist moth pollinators, *Epicephala* spp. Attraction to individual volatiles was lower compared to the mix of these two volatiles.[74]

In summary, both mechanisms (bouquets or key substances) find support in the literature showing that both modes of chemical communication can satisfy plant and/or animal needs. However, especially in more generalized systems, the data are biased towards key substances, which may also be the most parsimonious mechanism to explain how insects recognize, discriminate and learn floral cues/signals in an environment that is packed with other (often meaningless) volatiles. Because few studies have directly tested the importance of ratios of floral volatiles in mediating plant–animal interactions, conclusions about the generality of one or the other mechanism are premature.

**Signal *versus* cue:** traits displayed by an organism specifically to communicate with another organism (e.g. floral traits used in pollinator attraction) are called signals while cues are traits used by receivers but that are not intentionally displayed to attract their attention (e.g. floral traits used in host finding by herbivores).

## 3 Non-Volatile Flower Traits—Patterns and Functions

### 3.1 *Floral pigmentation*

In addition to scent, floral visual display is another trait whose components are perceivable by flower visitors from a distance. The visual display is composed of flower (and inflorescence) shape and size, but most importantly by floral pigmentation that results in various colours; less well investigated are floral iridescence,[75] and gloss[76] that results from epidermal cell shape.[77] Like volatiles, colours are used by flower visitors to find resources and by plants to guide mutualists into the rewarding floral structures or to avoid interactions with antagonists. Accordingly, animal taxon-specific preferences for colours can partition floral resources in plant communities with diverse floral colours,[21,78] reducing competition among flower visitors and facilitating flower fidelity. Particularly well investigated colour differences in bee *versus* bird-pollinated flowers often show consistent differences across plant families,[79] including reflection of ultraviolet (uv) light in red bee-pollinated flowers but not in bird-pollinated flowers but the opposite trend in white flowers.[80] The importance of plant species-specific colours is also suggested by studies showing that plants, at least those that are rare, diverge to a larger extent in floral colour from co-flowering plant species than expected by chance,[81,82] which is in concordance with the hypothesis that plants increase precise pollen transfer by unique recognition cues/signals such as colour. Colour discrimination by pollinators may select for colour divergence among sympatric plant species. For instance, *Phlox drummondii* flowers have higher pigment intensities when co-occurring with *P. cuspidate* than in populations where the related species is absent. Furthermore, stronger pigmentation in *P. drummondii* flowers leads to higher flower fidelity and thus reduces pollen transfer between species.[83]

The biosynthesis of pigments that are specifically attractive to certain groups of animals are hypothesized as an adaptation to pollinators. For example, in the plant family Polemoniaceae those species that are pollinated by hummingbirds contain pelargonidin and sometimes also cyanidin, whereas flowers pollinated by bees and beeflies are dominated by delphinidin.[84] Shifts in pigmentation resulting in floral visitation by different animal species may be encoded by alleles at a single locus, which suggests that a single mutation may be sufficient to switch pollinators resulting in sexual isolation from related species.[85] Unfortunately, information on pigment distribution across flowering plant species and their effect on flower–visitor interactions is limited. Much more commonly studied are colour characteristics such as hue, green contrast, brightness, saturation and salience emerging from an interaction between the colour receptor sensitivities of the flower visitors and the reflectance of ambient light by the flower structures as measured by a reflectance spectrum.[72,86–89] Flower visitors can have innate preferences for such visual characteristics or learn to use them to make associations with rewards. Honeybees, for example, have clear innate preferences for specific hues (bee-uv-blue and bee-green as defined by the colour-hexagon),[86] which may be an adaptation to those colours that are associated with flowers with the greatest probability of offering high levels of nectar rewards.[90] However, innate preferences can be modulated by learning[91,92] to allow flower visitors the flexibility to exploit the most rewarding of the currently available resources irrespective of whether they match innately preferred colours. Another less investigated function of pigments and resulting colours is the avoidance of visits by antagonists. Flowers may display colours that are hard to detect for antagonists,[80] or pigments can serve as antifeedants that reduce damage by florivores.[93–95]

**Innate preferences:** are the preferences of naïve animals for scents, colours and other flower traits that exist prior to the first contact with flowers.

## 3.2 *Floral morphology*

The basic structural plan of a flower includes both outer vegetative (calyx and corolla) and inner reproductive (androecium and gynoecium) parts. The vast diversity of floral form has long since fascinated biologists,[96] who often classified floral morphologies into broad categories. For instance, Müller[97] proposed a classification system for flower types that was related to the principal visitor (pollinator) of the flowers. However, including assumptions about the main flower visitor in a classification system may bias investigations of flower–visitor interactions by suggesting the main flower visitor or even pollinator before careful observations. The classification system by Kugler[98] mostly avoided the use of zoocentric descriptions of flower morphology, and provides a better basis for neutral investigations. Both classifications are still often used,[99] mostly to provide a preliminary impression about the overall appearance of the flower. These categorical descriptions are complemented by quantitative measurements of the flower's morphology, which is both more precise and neutral as well as allows for documentation of inter- and intra-specific variation in traits. Detailed measurements of nectar tube length, display size, and various other morphological traits are prerequisites for answering many questions about floral evolution and ecology. Most prominent are studies investigating pollinator-mediated selection on individual aspects of flower morphology such as spur length or flower display size,[100,101] which provide insights into how flower morphology evolved and how it affects plant reproduction. Instead of calculating selection gradients for each morphological trait individually, another line of research considers the overall architecture of flowers by relating different quantitative traits and testing for phenotypic integration.[102,103] It is useful to consider that flowers often have an integrated phenotype with fixed proportions between the dimensions of several morphological traits; such integration facilitates precision and thus efficiency of pollen transfer by pollinators that are forced to physically engage with flowers and to interact with reproductive organs as dictated by the flower's morphology.[104] Phenotypic integration is often particularly pronounced in flower traits directly related to pollen receipt and removal compared to morphological traits that attract pollinators[105] and in flowers specialized to a restricted taxonomical spectrum of pollinators with a narrow range of body sizes.[106] Furthermore, quantitative measurements of flower morphology are also used in the context of community ecology where floral structures are regarded as barriers that may either allow or prevent access to resources for different flower visitors depending on morphology.[19,21,107,108] For example, the fusion of petals can create unique architectural features such as tubular shapes and nectar spurs which limit the type of visitors that can access nectar, potentially leading to plant–pollinator co-evolution and diversification.[109] Additionally, flower shape, size and symmetry can function as a visual cue for pollinator attraction.[110,111] Finally, although individual flower morphology plays an important role in plant–animal interactions, the structure of inflorescences should not be ignored. For example, bees have been shown to have preferences for inflorescence architectural categories (e.g. panicles, racemes, and umbels) independent of other floral traits.[112]

### 3.3 *Floral rewards*

Animal pollination is often first characterised as a mutualism, although cheating by both parties is common. In the idealized exchange, plants provide rewards to animals for transport of plant gametes. Most commonly, nectar and pollen function as rewards for animal-pollinated species, although in specialized systems other rewards such as scent[113] and shelter are offered.[114] Plants can cheat in this exchange via food deception/rewardlessness,[115] sexual deception and oviposition site mimicry[40] which could result in variable rewards within an inflorescence or population.[39] While pollen is the male gamete of plants and thus has a primary sexual function, nectar is produced only as a reward in mutualisms, and could only be regarded as a secondary sexual character.

Nectar is a sugary solution used as fuel for pollinators[116] and non-pollinators.[117] Sugar content and type varies among species and with the preferences of the floral visitors.[118,119] Additionally, amino acids within nectar may also function as rewards.[120] Although a reward, nectar can also contain distasteful and even toxic compounds,[121] to manipulate pollinators,[122] repel nectar robbers[123] or to protect nectar from microbial growth.[124] Not surprisingly nectar rewards can influence flower visitation[125] and plant reproduction.[126] However, detecting selection on this important pollination trait has been challenging. The few researchers to measure selection on nectar frequently find no significant selection, e.g.[127] Nectar production may more likely experience stabilizing selection[128] and/or selection to increase signal honesty[38] because simply increasing nectar production may come with both physiological[129] and/or ecological costs.[126] Likely due to its highly dynamic nature,[130,131] difficulty of relating plant-mediated nectar amounts to standing nectar crops[132] and variability within inflorescences,[133] nectar has often been ignored in studies on floral ecology and evolution, e.g.[134] Nonetheless, some studies show that nectar sugar and amino acid composition as well as quantity reflect the requirements of the principal pollinators.[23,125,135]

Pollen can be a protein-rich resource.[136] For bees especially, pollen is an important food source, e.g.[137], although nectar is the principal resource for other pollinators such as birds and butterflies/moths. Bumblebees can discriminate between low and high quality pollen in the field[138] and honeybees exhibit distinct preferences for pollen types in the lab.[139] However, pollen protein content seems more driven by phylogeny than pollinator preferences[140,141] and pollen–stigma interactions are likely an important driver of pollen size.[142] While pollen traits of any species likely strongly reflect their sexual function, animal-pollinated plants with only pollen rewards produce more pollen than those with nectar[143] suggesting the additional importance of its reward function. Furthermore, since pollen is perceived by bees as a reward,[144] pollen can benefit plant female fecundity, independent of its sexual function by providing a reward to pollinators.[145] Also, the type of pollen presentation (simultaneous *versus* pollen dosing) can vary with the efficiency of the pollinators[146] suggesting a balance between its sexual and reward functions.

## 4 Interaction Effects of Volatile and Non-Volatile Traits in Flower–Visitor Interactions

In the previous sections, we discuss the importance of floral scents, colour, morphology and rewards in interactions between flowers and their visitors. From the numerous studies on each of the traits individually their ecological significance in manipulating flower visitor behaviour and influencing plant reproduction becomes clear. However, the multimodal display of flowers suggests strong interaction effects between volatiles and visual, tactile and gustatory traits (and also between the latter, which is beyond the scope of this paper). Information on these combined effects is available but not to the extent of each of the traits alone and will be discussed in the following sections. As a quick reference, the main outcomes of the additive and synergistic functions of volatiles in combination with other floral traits are summarised in Table 1.

***Table 1:*** Summary of interactions between scent and other floral traits.

| Effect | Examples |
|---|---|
| **Scent + colour** | |
| Pollinator attraction | • Pollinator visitation is best explained by a combination of scent and colour[72] (and morphology)[160]<br>• Innate attraction of hawkmoths to both visual and olfactory cues, but only the combination elicited proboscis extension and feeding[162]<br>• Visual and olfactory cues are used in combination by naïve and experienced specialist bees while foraging[163] |
| Pollinator learning/ flower constancy | • The combination of colour and scent facilitates learning by bumblebees faster than either trait alone[157]<br>• Floral constancy is increased by combinations of traits (e.g. colour + scent) than by colour alone[165]<br>• Odour differences can modulate innate colour preferences in hawkmoths[147]<br>• Scent differences reduce bumblebee uncertainty about colour variation in the lab suggesting a role for floral complexity[166]<br>• Foraging efficiency of hawkmoths increases when both olfactory and visual cues are presented in combination[164] |
| Back-up for variable environments | • Scent reduces loss of accuracy in low light for bumblebees trained on artificial flowers[167] |
| Biochemical pathway limitations/pleiotropy? | • Floral colour morphs do not consistently differ in scent, suggesting biochemistry does not explain scent/colour variation in *Hesperis matronalis*[168,169]<br>• Mutations to one anthocyanin gene (ray flower) but not another (white flower mutant) affect floral scent emission in *Ipomoea purpurea*[170]<br>• Scent and colour are sometimes related in the orchid *Calanthe sylvatica* but not for all colour morphs[154] |
| Mimicry | • In a fungal floral mimic, the importance of scent and visual cues depends on the visitors[171]<br>• Flowers use volatiles and colours to mimic their models[172]<br>• Floral scent but not floral colour determine pollinator visitation in *Ophrys arachnitiformis*, suggesting colour variation is not due to pollinator-meditated selection[155] |
| Protection/herbivore avoidance | • Both floral colour and scent predict seed predator damage in *Silene* $F_2$ inter-specific hybrids[173] |
| **Scent + morphology** | |
| Pollinator attraction | • Pollinator visitation is best explained by a combination of scent and morphology (and colour)[160] |
| Pollinator learning/constancy | • Olfactory cues in combination with flower shape increase foraging efficiency in bumblebees[174]<br>• Bumblebees are more constant when flowers differ in both scent and size than either alone[175] |
| Volatile dispersal/presentation | • Indirect evidence from humidity gradients created in headspace of funnel shaped flowers; floral morphology may provide similar gradients for scent emission[176]<br>• Tissue specific variation in scent production will create spatial variation in scent[177,178] (also see below for examples of spatial variation with nectar and pollen scents) |
| Functional synergism | • Trap flowers employ volatiles to attract pollinators that are forced by morphology to deposit pollen on the stigmas[179,180] |
| Mimicry | • Orchid species mimic volatiles and morphologies of female bees[63] |
| Context dependence/ defence trade off | • Flowers defend themselves either by repellent volatiles or by morphologies restricting access to rewards[181]<br>• Wider, more accessible flowers emit more repellent floral volatiles[19,37] |
| **Scent + reward: Nectar** | |
| Honest/dishonest signalling | • *Osmia* bees can 'smell' nectar in *Penstemon caesius*[182]<br>• Nectar scent is not correlated with nectar amount in *Polemonium viscosum*[37]<br>• Nectar scents are more similar among three beetle pollinated species than the rest of the floral parts[183]<br>• *Penstemon digitalis* nectar scent is uncorrelated with nectar amount |
| Nectar guides | • Spatial variation in scent of *Runuculus acris*,[184] *Silene latifolia*[177] and *Protea* spp.,[183] *Dianthus inoxianus*,[185] *Penstemon digitalis* (unpublished) |

(*Continued*)

***Table 1:*** (Continued).

| Effect | Examples |
|---|---|
| Pollinator learning | • Honeybees can learn scent from nectar received in the hive[186] |
| Context dependence | • Sugar concentration of nectar modulates responses to scents (see case study) |
| Pollinator manipulation | • *Nicotiana attenuata* nectar scents reduce visitation duration but increase number of visits, potentially enhancing outcrossing[187] |
| Protection/nectar robbers | • Nectar scents reduce ant visitation in *Nicotiana attenuata*[187]<br>• Floral scents repel ants[188]<br>• *Polemonium viscosum* nectar scents deter ant nectar robbers but also bee pollinators[37] |
| Protection/nectar microbes | • Volatiles in nectar may serve antimicrobial functions; direct evidence is lacking[189] |
| Deception | • Deceptive flowers attract pollinators using volatiles but provide no reward[151,179] |
| **Scent + reward: Pollen** | |
| Honest signalling | • Scents (discernible to the human nose) predict honeybee visitation to skunk cabbages but not pollen presence[190]<br>• Bumblebees use pollen odours of *Rosa rugosa* in visitation decisions[191]<br>• Pollinators avoid female plants that are missing pollen odours from their floral bouquet[192]<br>• Pollen odours are preferred by a pollen eating beetle in Y-tube tests[193]<br>• Pollen odours can be so variable that they are unreliable indicators of pollen chemistry[194] |
| Pollinator learning | • Honeybees can learn to associate pollen odours with reward[144,193,195]<br>• Solitary bees use pollen odours in foraging and prefer the odour of pollen they were reared on, although their behaviour is modified by experience[196] |
| Defence against herbivores | • Specialist bees fail to develop on alternative pollen, suggesting that pollen contains defensive compounds that may or may not be volatile[197] |
| Pathogen defence | • Floral scents protect stigmas (and pollen) from bacteria[198,199] |

### 4.1 *Interactions between volatiles and colours*

When a sender presents two or more cues to a potential receiver, the presence of one cue may either modulate the function of the other, or both cues may act additively or synergistically towards a common function. For example, in the moth *Macroglossum stellatarum*, the strong innate preference for blue flowers prevents these insects from discriminating volatiles if they are presented on blue artificial flowers, a task that is well performed when the volatiles are presented in the context of less preferred colours.[147] Likewise, the responses towards artificial flowers presenting olfactory and visual cues differed in moths that were visually impaired and those with full visual abilities[148] showing the importance of both visual and olfactory cues/signals in animal behaviour.

Biosynthetically, some volatiles and pigments are related to each other since some pathways synthesizing volatiles and pigments share precursors: monoterpenes and carotenoids (yellow, orange and red pigments) both derive from the pyruvate pathway; phenylalanine is the common precursor for aromatic compounds and anthocyanin pigments (blue, violet and red). Correspondingly, it has been hypothesized that specific scent/colour combinations in flowers may not only result through parallel selection by pollinators, but from pleiotropy.[149] In support of this hypothesis, Zvi *et al.*[150] found that an anthocyanin regulating transcription factor also up-regulates the production of aromatic volatiles. Other studies either have[151,152] or have not[148,149] found similar patterns, e.g.[153,154] Furthermore, the behavioural significance of biochemical connections is questionable. A study testing learning success in bumblebees when confronted with combinations of either anthocyanins + benzenoids or carotenoids + monoterpenes found no differences compared to combinations of pigments and volatiles derived from different pathways.[72] Thus, selection pressures may not act on specific pathways or the shared precursors of scents and pigments – within potential limits of pleiotropy – but rather on the overall (pathway-independent) attractiveness of the flowers resulting from the olfactory and visual phenotype. However, this hypothesis needs further validation.

Studies gauging the relative importance of volatiles *versus* colors in flower–visitor interactions have had mixed results. While some studies show a dominant role of floral volatiles,[155] others found flower colour to have a stronger impact than scent on flower visitor choices.[156] The single study to measure natural selection on both colour and scent found stronger selection on scent in *Penstemon digitalis*[55] but the generality of this finding is unknown. Studies showing a prominent role of volatiles *or* colour are complemented by a large number of studies showing the combined (additive or synergistic) function of volatiles *and* color in controlling flower visitor behaviour. For example, in the lab, both the learning success of rewarded artificial flowers as well as initial responses of bees to them were better explained by the combined olfactory and visual salience of a stimulus than by olfactory or visual salience alone.[72] Similarly, bumblebees challenged with discrimination tasks succeeded best if both olfactory and visual cues were presented compared to trials where only one trait was available.[157,158] These lab-based findings are mirrored in field studies showing that scent and colour jointly affect flower visitation frequencies by animals.[159–161] Several species of the family Passifloraceae have diverged in their olfactory and visual cues (and also nectar characteristics) to adapt to the pollination by bats, birds or bees,[23] which clearly shows that these traits are exploited in combination by plants to attract their specific pollinators and thus to increase reproductive success.

**Floral antagonists:** are organisms that have a negative net effect on plant reproduction when visiting flowers including nectar thieves/robbers, florivores, pollen thieves, predators of pollinators and microbes that spoil floral rewards or cause disease in floral tissue.

## 4.2 *Interactions between floral volatiles and morphology*

Unlike responses of animals to the combination of flower volatiles and colours, the combined roles of volatiles and floral morphology in floral and pollination ecology have rarely been specifically addressed. The spatial patterns of scent production have been examined, showing that different flower parts emit different volatiles,[183,184,200] which has been interpreted as olfactory nectar guides analogous to visual nectar guides.[177] Similarly, pollen-collecting animals may use volatiles specifically emitted by nectar or pollen to find these resources,[201–203] (see also section on resources and volatiles). For volatiles produced in floral petals, visual and scent cues may be linked if the area of the petal affects both the size of the visual cue and the amount of scent produced. Some flowers rely on the interplay between volatiles and morphology to mimic models in shape and scent[63] or to lure pollinators by volatiles into trap flowers where they are forced to deposit and remove pollen.[179] More general examples include systems where both morphology and scent determine the visitors on flowers.[160,204] Furthermore, floral shape can influence the importance of floral volatiles. For example, *Polemonium viscosum* flowers that are more open and thus more likely to experience nectar robbery by ants also tend to have higher emissions of repellent floral volatiles.[37] Such effects are viewed as trade-offs between volatiles and morphology in protecting flowers against floral antagonists. Accordingly, across numerous plant species[19,181] or within a species,[37] it has been shown that flowers either display morphologies that restrict ant entry into flowers or emit volatiles that repel ants. One unexplored option for an interaction between morphology and scent is that tubular flowers may concentrate scent within the tube or create a scent gradient in the headspace around the flower. A similar process has been shown for humidity gradients produced from nectar evaporation in tubular flowers;[176] however, this hypothesis has not been tested for scents.

## 4.3 *Interactions between floral volatiles and rewards*

Both pollen and nectar rewards can be presented openly to visitors where the presence and abundance of reward can be assessed prior to landing. However, rewards are often cryptic/hidden within the flower necessitating either direct assessment of the reward through tactile and gustatory sensing or by forming learned associations with more easily assessed cues such as scent to aid in foraging efficiency. The implicit assumption in the study of floral cues is often that these are informative for floral visitors regarding the rewards present (except in deceptive systems).

Directly scented rewards may provide honest signals to pollinators of either the presence of nectar[203] or pollen,[201] however the whole floral bouquet is likely to be more loosely associated with reward status as occurs with the cues of floral colour or size. The overall floral scent may help distinguish between species and aid in learning rewarding plants, but it does not necessarily provide a direct cue of the current amount of reward on a particular plant or flower. Theoretically, although nectar or pollen scents should provide honest cues of reward presence, the relationship is not that close in some systems,[37] suggesting that these scents diffuse from petals/other floral parts into the reward rather than being produced along with it. However, because these scented rewards can be both smelled and tasted by foragers, they may provide particularly potent cues. In laboratory settings pollinators can quickly learn to associate scents with nectar rewards[62,205] and nectar scents can be learned within honeybee hives, prior to an individual's foraging bouts.[186] Bees may also be capable of detecting floral nectar by scent[182] and even spiders can learn nectar odours after feeding on nectar.[206] Furthermore, as shown in the case study below, the reward level can modulate the dose-dependent responses to volatiles.

Pollen odours can have similar functions; pollen odour can influence bumblebee behaviour and foraging,[191,207] be learned by honeybees,[144,195] as well as used to distinguish rewards by solitary bees[196] and non-pollinating pollen foragers.[208] Pollen scents can drive differences within species with separate sexes, e.g. in gynodioecious strawberries,[192] although understanding patterns of scent in dioecious plants is still preliminary.[209] Beyond direct associations between scent and rewards, as mentioned in connection with morphology, scents may also play a role in orienting pollinators towards rewards via scent gradients (Table 1).

Although associations between nectar and pollen with scents may function to attract reward-seeking pollinators, these scents may also function to deter robbers of these resources or to provide resource protection. For example, in *Nicotiana attenuata*, nectar scents reduce visitation by nectar robbing ants but also manipulate pollinator behaviour by reducing visit duration while increasing visit numbers, thus potentially increasing outcrossing rate.[187] For *Polemonium viscosum*, ant repellence of nectar volatiles may come at the expense of visitation by their bee pollinators.[37] Although pollen volatiles may also provide protection, this is an understudied aspect of floral biology. However, floral scents do protect stigmas (and thus also pollen) from bacteria in *Arabidopsis thaliana*,[198] suggesting the possibility for defensive roles of pollen volatiles.[202]

### 4.4 *Case study: Pollinator responses to volatiles are modulated by reward levels*

Few studies have tested whether pollinator responses to volatile cues are fixed across different conditions, or whether these responses are flexible and dependent on other floral traits such as rewards,(butsee 2010). In a field experiment, we tested whether and how different reward levels (sugar concentration of artificial nectar) modulate honeybee responses to varying concentrations of volatiles. A watery sucrose solution (7.5, 15 or 30 mass%) was offered in 2 ml standard reaction tubes attached to plastic rods 60 cm above the ground and arranged in a circle (diameter 1 m) with constant distances between them. Discs of blue foam rubber (diameter 4 cm) surrounded the tubes serving as the visual cue. In each trial, eleven artificial flowers of the same sucrose concentration were treated with different scent concentrations ranging from 0 to 3.84 mM (0, 0.01, 0.02, 0.04, 0.06, 0.12, 0.24, 0.48, 0.96, 1.92, 3.84 mM) of either methyl salicylate (Sigma-Aldrich, >99%) or linalool (Merck, >97%). The scent concentrations were chosen to match the concentration of volatile substances in nectar.[211] For each sucrose concentration ($n = 3$) and scent compound combination ($n = 11$ concentrations per trial, $n = 2$ volatile compounds), we performed $n = 10$ trials at different locations each (>100 m apart from each other, $n = 60$ trials) within the Botanical Garden of the University of Düsseldorf, Germany (Fig. 2A). We observed the eleven artificial flowers for a one minute duration six times within a 30 min period and counted the number of honeybee foragers per artificial flower, resulting in 360 min total observation time across all trials. For each flower type within a trial, we calculated the proportional deviation from equal visitation (total number of visits per trial/11 flowers) to indicate either a positive or negative effect (positive/negative deviation) of the scent concentration presented. We analysed the data with a linear mixed-effects model (lmem) with sugar concentration, scent concentration and scent compound as fixed factors, trials as random factors and deviation from equal visitation as the response variable. Trials with no honeybee visitation ($n = 5$ trials) were excluded from statistical analysis.

In total, we observed 5,019 honeybees visiting the 605 artificial flowers in 55 trials. As we did not mark bees, we cannot exclude the possibility that individual workers returned to artificial flowers within or across individual trials. However, return visits were more likely to affect the result within trials and thus trial was included as a random factor in the statistical analysis to account for this possibility. On average, honeybees did not respond differently to the two scent compounds (Fig. 2C) or the three sugar concentrations (Fig. 2C), whereas scent concentration strongly affected honeybee choice (Fig. 2B and C). Interestingly, interactions between scent compounds and scent concentration (Fig. 2B and C) as well as between scent concentration and sugar concentration (Fig. 2B and C) had a strong effect on honeybee foraging decisions (Fig. 2B and C). Decisions based on scent concentration were more pronounced in trials with low sugar concentrations than those where bees were rewarded with higher sugar concentrations. This effect is shown by the low absolute values of deviation from equal visitation to all artificial flowers in trials with 30% sucrose solution and by the negative correlation between sugar concentration and variance of proportional deviations from an equal distribution (Spearman's rank correlation: $r_s = -0.84$, $p = 0.038$, Fig. 2D).

Our results demonstrate that, similar to nectar toxins,[212] rewards can modulate bee behaviour towards floral volatiles, suggesting that responses to floral scent compounds can be flexible and dependent on the perceived or expected reward. Trials where 15% sucrose solution was offered to honeybees confirmed the dual role of volatiles in both serving as attractant and as repellent[4,53] and that these functions depend on volatile concentration.[37,213] The responses of honeybees as a function of scent concentration were different in trials where the reward concentration was altered. When honeybees were rewarded with very low levels of sugar, their preference shifted towards very low concentration of scents suggesting that they now avoided scent concentrations that they tolerated when the reward was higher. The tolerance of volatiles that are usually avoided in lower concentrations is also indicated in trials where honeybees were allowed to consume a 30% sucrose solution: in these trials the distribution of honeybees at artificial flowers containing different scent concentrations deviated little from an equal distribution. The finding that higher scent concentration act as deterrent supports results by Galen *et al.*[214] who showed that high concentrations of 2-phenylethanol repelled both ants and bumblebees, whereas lower concentrations of the same compound were behaviourally insignificant. Furthermore, our finding is concordant with the hypothesis that primarily defensive volatiles can be used as attractive signals/cues by obligate flower visitors that adapted to tolerate these substances.[4] We conclude that besides the finding that reward levels can shift preferences for scent concentrations, our results suggest that the importance of volatiles in flower choices decreases with increasing reward levels. Both findings deserve a validation in further field or lab-based studies.

## 5 Statistical Approaches to Deal with Multimodal Phenotypes

In studies testing insect responses towards artificial flowers with experimentally designed trait combinations, statistical analyses are straightforward including correlations, students *t*-tests, or generalized linear models. However, in field studies where the diversity and distribution of multimodal characteristics of floral phenotypes are documented, different, more sophisticated, statistics are required. A full review of methods that have been applied to deal with multivariate data obtained by quantifying floral phenotypes is beyond the scope of this paper, but we will highlight some approaches and offer some recommendations to start with.

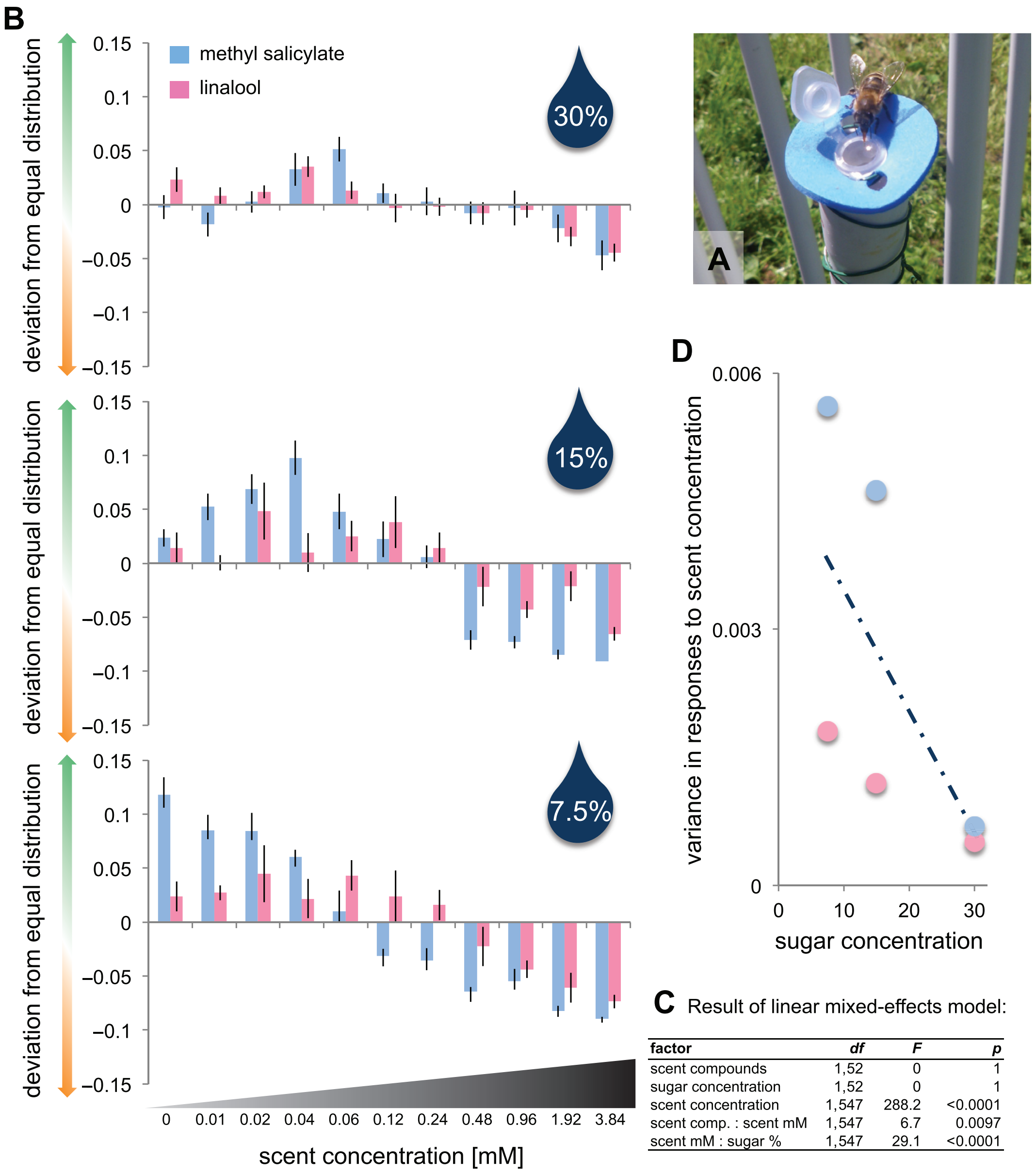
### 5.1 *Data structure*

Although qualitative descriptions of floral traits have been used for centuries, quantitative measures, preferably on the ratio scale, are necessary for detailed studies (e.g. the description of a sweet scented purple flower cannot be treated in any meaningful statistical manner). Scent bouquets are a multivariate trait that can be described by emission rates ($ng \cdot h^{-1}$) of individual substances. Nutrient composition (e.g. sugars and amino acids) or secondary metabolites (volatiles, alkaloids, phenolics) in nectar and pollen can be combined in the same multivariate data structure. Often, nectar amount ($\mu l \cdot flower^{-1}$) and sugar concentration (% sugar) are given as univariate factors; the same is true for morphological measurements (nectar tube depth, stamina length etc.). In these traits (secondary metabolites, morphology, nutrients), each factor (i.e. each component or each morphological characteristic) can be of importance by itself or in combination with others. The situation is different for floral reflectance responsible for floral colour, which is expressed as a function of wavelength (see Fig. 1), or more practicably as the reflection of light for each integer of wavelengths between 300 and 700 nm. Here, each value by itself is more or less meaningless as the colour impression results from the whole spectrum, not from individual wavelengths. As a result, reflectance spectra are often translated into univariate characteristics such as hue, green contrast, brightness, saturation and salience,[72,86–89] which are sometimes applicable for few taxa only (e.g. the colour hexagon is specifically designed for honeybees)[86] or represent simplifications with sometimes little explanatory power for insect behaviour. Thus, one challenge of the statistical treatment of multimodal phenotypes is the varied types of data describing the different modalities of the floral phenotype.

### 5.2 *Character-wise treatment*

**Phenotypic selection:** a within population measure of the relationship between traits (standardized) with relative fitness. The form of selection can be linear or non-linear and is often estimated as either total selection on a trait (differentials (S), from univariate regression models) or direct selection on a trait (gradients (β), from multivariate regression models that control for correlations among traits).

In studies of phenotypic selection, each quantitative trait is used as an individual factor in multiple regressions that quantify selection.[215] Researchers use multiple regressions of traits on relative fitness (individual fitness relative to the population) that account for correlations among traits. These selection gradients can identify the



**C** Result of linear mixed-effects model:

| factor | *df* | *F* | *p* |
|---|---|---|---|
| scent compounds | 1,52 | 0 | 1 |
| sugar concentration | 1,52 | 0 | 1 |
| scent concentration | 1,547 | 288.2 | <0.0001 |
| scent comp. : scent mM | 1,547 | 6.7 | 0.0097 |
| scent mM : sugar % | 1,547 | 29.1 | <0.0001 |

***Figure 2:*** Responses to volatiles are modulated by reward levels. Honeybees chose between artificial flowers offering watery solutions containing variable scent concentrations and variable sugar concentrations. (**A**) Experimental design of the bioassay with honeybees. An artificial flower offering a watery sucrose solution treated with scent is shown. (**B**) Bars denote deviations from an equal distribution (mean ± SE) of honeybees on each of the artificial flowers. In each test, eleven artificial flowers were offered containing a watery solution with a constant sugar concentration (7.5, 15 or 30%) but a variable scent concentration (0–3.84 mM, methyl salicylate or linalool). Honeybee visits were counted within a period of 30 minutes and deviations from a equal number of bees per artificial flower were calculated. Positive deviations indicate more visits than expected, negative deviations indicate less visits than expected. (**C**) Results of the linear mixed-effects model testing the effect of scent compound, scent concentration [mM], sugar concentration [%], and interaction terms on the deviation from an equal distribution of honeybees to artificial flowers. (**D**) Relationship between rewards level [sugar concentration %] and the variance in the responses to scent concentration. The lower the reward level the higher was the importance of scent on the bees' choices.

targets of selection for individual morphological traits[100] or scent compounds,[55] although they can only account for the traits included in the model (i.e. the target of selection may be some unmeasured trait). For measures of selection, traits are standardized to deal with the problem of differences of scale among traits. When designing studies, it is important to note that in multiple regressions the number of factors should not exceed a third of the number of samples;[216] this is especially relevant if flowers emit a large number of scent compounds. For a full discussion of how to handle data from complex phenotypes in selection studies including visual, morphological and scent data, see Parachnowitsch *et al.*[55] Briefly, researchers can reduce traits included in statistical models by creating meaningful composite traits (e.g. using PCA values, geometric means of floral dimensions, or total scent emission) or by selecting a subset of traits based on trait correlations and univariate models. The following section discusses further how to reduce trait complexity and results of these approaches can then be used within the selection framework.

### 5.3 *Reducing the complexity—multivariate statistics*

Matrices containing the quantities of compounds emitted from flowers or different morphological characters of each sample can be analysed using ordination methods such as non-metric multidimensional scaling (NMDS) and principal component analysis (PCA). These methods allow for graphical evaluation of the similarity of flowers based on multivariate data and also reduce the number of variables as in PCA. Such procedures are commonly used for both scent profiles and complex morphological datasets and can be conducted with the help of the *vegan* package[217] for the statistical software R[218] that comes with a useful tutorial (available at http://vegan.r-forge.r-project.org/). A nice example of how measurements of multiple morphological traits can be summarized using ordination is given in Chartier *et al.*,[219] but their approach can easily be adapted to other traits.

Relatively recently, ecologists started to use 'Random Forest', a machine learning algorithm[220] after the suggestion of its application from Ranganathan and Borges[221] for scent bouquets. In short, Random Forest classifies samples based on multivariate data and reports the most important factors (i.e. volatiles, morphological characters etc.) for the discrimination between groups; for an example of its applicability, see.[51] Random Forest is also implemented for R in the package *randomForest*.[222]

As a quick note on how to deal with and visualise floral reflectance data, we refer to the excellent *pavo* package for R,[223] which offers numerous functions to transform reflectance data into ecologically meaningful values. Furthermore, the *stimulation landscape* as proposed by Renoult *et al.*[224] may be an interesting approach addressing the need to consider the diverse visual systems of animal taxa interacting with plants. However, to our knowledge, the suitability of this approach has not been tested for a broad range of systems, preventing conclusive statements.

### 5.4 *Trait space approaches*

Ecologists working on multimodal floral phenotypes may adopt statistical procedures from niche theory and functional diversity research. The phenotype of flowers can be seen as a $n$-dimensional trait space based on $n$ quantitatively defined traits. The functional richness of plant populations (intraspecific variation) or of plant communities (interspecific variation) can be estimated by defining the minimum convex hull volume that includes all of the samples belonging to a group.[225] It is suggested that the trait values are standardised prior to the analysis to make sure that all traits are weighted equally.[225] Approaches like this may, however, relatively quickly reach their limits if too many traits are considered, which is likely when including scent bouquets. One approach to deal with many factors is to work with distances between samples (e.g. Euclidean distances) based on either individual characters (morphological characters or individual scent compounds) or on multivariate traits (e.g. scent bouquets or reflectance spectra). An advantage of this approach is that traits with different data structures can be integrated into a single statistical analysis. On the down side, the position of individual samples within a trait space cannot be independently identified because their phenotypes are defined relative to the other samples. Nonetheless, distance based indices such as Rao's quadratic entropy, Rao's Q[226] of species or communities are widely used and are well suited to characterise multimodal phenotypes of flowers.[21]

Note that the examples mentioned above are far from complete; additional methods have been used to statistically treat multimodal phenotypes or can be adopted from other disciplines. However, we present a range of potential approaches to deal with large datasets which will hopefully stimulate future efforts to apply novel approaches to these complex data.

## 6 Why Do Flowers have Multimodal Displays?

The most obvious answer to the question of why flower displays are multimodal is, as previously discussed, that floral characters can have multiple functions. Thus, in some respects we may intuitively expect flowers to have multimodal displays because of their complex interactions with mutualists and antagonists. The vast majority of flowers are animal-pollinated[227] and plant–pollinator interactions are thought to be the major drivers of floral diversity.[13,35] However, florivores,[228] nectar robbers,[117] predispersal seed predators,[229] and microbes[230] can also interact with flowers and that is not even taking into account whole plant abiotic and biotic interactions that might also influence flowering and floral characters.[231,232] Thus pollinators and non-pollinators may impose selection pressures on the same or different components of the floral display and thereby drive the evolution of complexity. Moreover, floral characters also need to be considered within the context of the whole plant.[48] For example, interactions with herbivores may alter pollinator–interactions[233,234] suggesting that correlational selection may act on trait combinations and explain complexity of floral displays.

There may also be advantages to maintaining complexity within the same function, as in the case of multimodal cues.[235] For example, when signalling to pollinators, different aspects of a floral phenotype may be important under different conditions. Night blooming flowers pollinated by bats, moths, and beetles have long been known to produce strong floral scent bouquets with less reliance on floral colour to attract their pollinators.[236–238] Similar processes could also determine floral complexity in day blooming plants when light conditions are poor; scent may provide an alternate signal to colour in low light conditions[167] and likewise, colours may provide information when scent plumes are difficult to detect due to wind conditions.[239] As discussed in the context of floral scent bouquets, if pollinators only need one or a few key substances for attraction and learning, why are floral bouquets so complex? One answer may be that pollinator behaviour is often studied in controlled lab conditions and understanding foraging in a community context could help explain the complexity of floral scents.[7] Another possibility is that different key substances mediate the many interactions flowers experience, ranging from mutualists to antagonists, as suggested by the private channel hypothesis.[7] In general, complex signalling may offer advantages to more simple communication and help explain why flowers are such complex structures.[235]

Pollinator learning and behaviour may drive the evolution of floral complexity. Pollinator constancy (visitation of the same flower type when other rewarding flowers are available) is best achieved by complex phenotypes[165,175] presumably because multimodal floral displays push the limits of pollinator memory. It has been suggested that when displays are complex, pollinators can only keep a single flower type in active memory and thereby stay constant on that species during foraging but for simple flowers differing in a single trait, pollinators can remember and visit multiple rewarding flower-types.[1,11] Alternatively, the need for multiple signalling modalities has been suggested to arise from different channels for communication with multiple visitors (e.g. mutualists and antagonists).[7] Some bumblebee individuals may be better at discriminating flowers across all modalities (colour and shape within visual channels as well as olfactory cues) than others suggesting that these multiple axes of floral variation are unlikely to provide different channels of discrimination, at least for bees.[240] However, different aspects of the floral phenotype could be used to mediate interactions across a range of species that respond to different sets of floral traits.[21] For example, insects with a long proboscis are less restricted in their flower choices regarding the depth of the nectar receptacles than insects with a short proboscis.[241] The long proboscis bearing insects that are not restricted in their accessibility to nectar may base their flower choices—or may be controlled by the flowers—by other traits such as scent, colour, rewards or other morphological traits.

Across traits, the state of one trait may also determine the relevance of another, either through trade-offs or context dependence (see case study above). For example, flowers may either restrict access to their rewards through morphology or protect easily accessed rewards with repellent compounds.[19,37,181] However, constraints on one aspect of the floral phenotype (either biotic or abiotic) may set a context for evolution of other aspects of the floral phenotype. For example, flowers with large flowers lose more water which can have profound effects on allocation to reproduction in drought conditions.[242] Whether a plant is rewarding or deceptive can impact other aspects of the phenotype, although it is difficult to determine causal links in the evolution of these traits; i.e. does rewardlessness evolve prior or in concert with the rest of the floral phenotype? However, rewarding species can rely on pollinators learning the relationship between cues and rewards to ensure pollination while deceptive species must avoid such learning to be successful. Thus understanding the whole flower context can provide information about the function of particular traits.

Complexity of floral display may also have an advantage because traits differ in their temporal variability. For example, some flower traits may be better suited to flexibly signal the pollination status of a flower. After pollination, many flowering plant species change their colour or scent emission[29,32,243] to either avoid pollen clogging, removal of pollen from the stigma or to allocate more visits to unpollinated flowers of the same individual. Flower closure has also been reported to quickly respond to pollination,[244] but it is possible that changes in scent emission may be even more immediate. Alternatively, relatively constant traits (e.g. scent, colour, shape that are stable for at least a considerable period of time) may disguise strongly variable resource levels either due to consumption or because plants may reduce costs for rewards,[245] which enables the plants to receive visitation despite strong inter-floral differences in rewards. Thus a multifaceted floral display may be used to maximize communication with and/or manipulation of flower visitors.

## 7 Future Perspectives

In the previous sections we have presented multiple cases where different floral traits interact with each other and where studying them in concert can provide additional insights compared to studies on traits in isolation. In particular, floral scents have often been studied separately from other aspects of the floral phenotype, especially in natural populations, perhaps in part due to the specialized training and equipment needed to identify and quantify volatiles.[246] However, we have highlighted many examples of studies that combine classical pollination/floral ecology with scent research in this review (summarised in Table 1). Beyond curiosity-driven motivations, these studies can provide real insight into floral diversity and evolutionary trajectories of floral phenotypes.

From the literature summarised in this and other review articles,[6–10] it becomes clear that floral scent bouquets are both chemically and functionally extremely diverse. The functional diversity ranges from attractive to repellent functions addressing generalised and specialised arthropods, vertebrates and microorganisms that respond either innately or after associative learning to key substances or whole bouquets. These comprehensive functions of volatiles are always complemented by equally diverse functions of floral colour, morphology and resources suggesting multiple selective forces shaping these complex phenotypes. Actually, none of these traits acts alone in flower–visitor interactions; rather they affect each other in multiple ways. Thus to understand floral diversity, function and evolution, investigations considering two or more traits are required to fully disentangle the various potential interactions.

Future studies should address questions about the combined effects of volatiles and other floral traits to elucidate the ecology and evolution of complex floral phenotypes and their effects on interacting organisms. Recently, studies on flower–visitor networks incorporated multiple functional traits into attempts to explain the network structure found in natural communities and have shown that multiple traits are important, suggesting that single traits alone cannot fully describe these networks.[21,247–251] However, none of these studies included floral scents (but see Junker *et al.*,[3] where no other traits were included apart from responses to flower volatiles by insects), suggesting an important gap in knowledge.[8] Integrating a full set of floral traits may help us to understand the structuring mechanism of communities and to gain insight into processes shaping and protecting biodiversity. Within the context of community ecology, similar studies may also provide information on the relative importance of each of the traits in determining network structure and the reproductive success of plants. Knowledge of the function of multimodal floral phenotypes could also be integrated into crop sciences, where cultivars with optimal trait combinations could be cultivated to promote interactions with mutualists while reducing negative effects of antagonists.

Multifaceted floral phenotypes may also be important for understanding natural selection and floral evolution. From an evolutionary perspective, it will be important to know the genetic and functional correlations among traits and to learn whether constraints prevent independent trait evolution. Questions in this context include whether pleiotropic effects or shared selection are more common in integrated phenotypes or whether specific combinations of traits (e.g. scent and colour, morphology and resources) are more likely to be correlated and jointly fulfil functions in interactions with mutualists and antagonists than others. Although there is a broad literature of selection on floral traits,[252] floral scents have generally been ignored.[57] Nonetheless, floral volatiles can experience phenotypic selection in field populations[54,56] and may even exhibit stronger selection effects than more commonly measured traits such as floral size and colour.[55] Selection studies that include many components of the floral phenotype have the advantage of elucidating the true targets of selection. Correlation selection, i.e. selection on particular combinations of traits, is thought to be common in nature[253] and estimating correlational selection should be an important goal of floral evolutionary ecologists. Although a few studies show that mechanical traits and/or visual signals can be functionally integrated and under correlational selection, e.g.[254–256] correlational selection should also act across sensory modalities within floral signals such as visual and olfactory traits. Whether correlational selection, across multiple aspects of floral phenotypes including scent, is common remains an open question.

We conclude that the complexity of floral phenotypes characterised by scents, colours, morphology and rewards offers manifold perspectives to study the ecology and evolution of species interactions that are mediated by these traits in an orchestrated way.

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