The ecology and evolution of pollen odors

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Abstract. The literature is reviewed and new evidence presented that pollen produces odors, which serve multiple functions in pollination and defense. Pollen odor, which originates from pollenkitt, comprises volatiles that belong to the same chemical classes found in flower scents, that are in species-specific mixtures, and that contrast with odors of other floral parts. Pollen can also take up volatiles from surrounding floral odors, but this adsorption is selective and varies among species. Pollen odors are more pronounced in insect- than bird- or wind-pollinated plants, suggesting that volatile emission evolved in part under selection to attract pollinators. Pollen-feeding insects can perceive pollen odor and use it to discriminate between different pollen types and host plants. Pollen odor influences bee foraging, including the location of pollen sources, discrimination of flowers with different amounts of pollen, and host-plant recognition by pollen-specialist species. In the few wind-pollinated plants studied, odors of male flowers or pollen are comparatively high in α-methyl alcohols and ketones; these volatiles may serve in pollen defense, with some known to repel insects. Pollen odor often includes chemicals with documented defense activity, which is probably aimed mainly at nonpollinator pollen-feeding insects and pathogens; an involvement in pollen allelopathy is also possible. Pollen volatiles comprise chemically diverse compounds that may play multiple roles, and their emission in pollen odor undoubtedly evolved under the principle, and often conflicting, selective pressures to both protect the male gametophyte and increase its dispersal by animals.

Key words: Pollenkitt, volatiles, flowers, plant defense, pollen foraging, pollination.

Following the heightened interest in pollination spurred by the publication in 1793 of Sprengel's "Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen", pollen received increased recognition for its role in pollinator attraction and plant reproduction (see Wodehouse 1935), but no mention of pollen odor appeared in the literature until the 1920's. Odors arising from anthers or pollen are often considered to represent the oldest food attractants for flower visitors (Crepet 1983) and to have preceded other olfactory and visual cues (Porsch 1954, Faegri and van der Pijl 1979), although floral secretions may have played the central attractive role in flowers that offered rewards other than pollen (Endress 1994a). Pollen odors most likely evolved as a defense against pathogens and pollen-feeding animals, prior to the development of animal pollination, following an evolutionary scheme suggested by Pellmyr and Thien (1986). As flowering plants became dependent on animals for pollen transfer, there would have been increasing selection pressure for pollen odor to include

volatiles attractive to the pollinators, which may have included both food-related and mating-related chemical cues.

Pollen of many species has since been described as having distinctive aromas as evaluated by the human nose (von Frisch 1923; Porsch 1954; 1956; von Aufsess 1960; Buchmann et al. 1977; Faegri and van der Pijl 1979; Buchmann 1983). In some plants, including mainly beetle-pollinated taxa in the Magnoliaceae (Thien et al. 1975) and Ranunculaceae (Pellmyr et al. 1984) and bee-pollinated taxa in the Solanaceae (D'Arcy et al. 1990), floral aroma is produced exclusively by the androecium, but the contribution of pollen in these examples still remains to be established. Meanwhile, over the last 10 years chemical analyses of volatiles in pollen headspace (i.e. air surrounding the pollen) in a dozen species have confirmed that angiosperm pollen has species-specific odors and that these are chemically distinct from the odors emitted from other floral parts (Dobson et al. 1990, 1996; Bergström et al. 1995). The chemical confirmation that pollen emits odors has given new impetus to studies of the role of pollen odor in pollination ecology, especially in attracting flower-visiting insects and modulating their foraging behavior, and in defending pollen against pollen-feeding organisms and pathogens (Dobson 1994, Dobson et al. 1999).

Perhaps most important in our attempts to shed light on the evolutionary significance of pollen odor is the need to keep in mind the two simultaneous conflicting pressures faced by the plant with regard to its pollen, namely the need to both protect it from over-exploitation by non-pollinating insects and pathogens and to advertise it as an attractive reward to pollinators. It is also possible that some of the volatiles may play a role in pollen-pistil recognition or interfere with such interactions through allelopathic effects (see section on defense). Pollen odor undoubtedly serves multiple functions, with the emission of individual components having evolved under different selection pressures. The advantage to plants in having their own particular pollen odor varies

with each plant's reproductive biology and needs to be evaluated on a species-by-species basis.

Pollenkitt as the source of pollen odors

Knoll (1930) was the first to address the origin of pollen scents and insightfully proposed that pollen odor arises from the pollenkitt. Knoll coined this term for substances that cause pollen grains to clump, more specifically in reference to the oily and often sticky, colored coating on the outside of pollen grains. Up to then, pollenkitt had attracted only spotty attention. Observations that pollen grains are enveloped in an oily coating were made by early students of pollen biology (Kölreuter 1761, Fritzsche 1837, von Mohl 1852). In the later 1880's, Fischer (in Wodehouse 1935) and especially Kerner von Marilaun (1898) recognized the role of pollenkitt in increasing pollen adhesion during pollination by animals, a function emphasized with the renewed interest in pollination biology in the 1920's (Parker 1926, Troll 1928). Knoll (1930) devoted an extensive discussion to the various functions of pollenkitt, the most notable being that it enhances pollen adhesion, provides yellow color that visually attracts flower visitors, serves as a valuable nutritious substance to pollen-feeding insects through the fatty oils it contains, and, most important here, that it contains the pollen odor. Indeed, Knoll appears to be the first to suggest that substances constituting the basis of pollen odor, which he points out to be of utmost importance to insects in locating and collecting pollen, reside in the pollenkitt. In the 1950's, pollenkitt oils were proposed to be located externally to the pollen protoplasm (Steffen 1953), which was confirmed with the development of electron microscopy (Pankow 1958). In subsequent extensive studies, Hesse (1980, 1981) established that while pollenkitt appears to be universally present in angiosperms, it varies in its distribution on the pollen surface, thereby conferring varying degrees of stickiness in association with different pollination

strategies. Pollenkitt is currently considered to play a multitude of functions besides providing pollen odor and olfactory attractants to pollinators (Dobson 1989, Pacini and Franchi 1993), including: protecting the male gametophyte against ultraviolet light, desiccation, and pathogens; aiding in pollen clumping and adhesion to animal vectors; providing visual attractants, as well as nutrition and phagostimulants to pollinating animals; and participating in pollen-pistil interactions following pollination (e.g. pollen adhesion to stigma, pollen hydration, germination, and pollen-tube growth).

Pollenkitt is thought to contain most of the lipid diversity in pollen, and thus to comprise the majority of non-polar ether-extractable materials, which represent 1–20% (but usually ≤5%) of the pollen dry weight (Barbier 1970, see Stanley and Linskens 1974). In anemophilous taxa (e.g. Poaceae), the values typically fall below 2% (Wittgenstein and Sawicki 1970, Bianchi et al. 1990). However, unless the extraction is very rapid (less than 30 seconds) to exclude the removal of internal lipids (Knox and Heslop-Harrison 1970, Heslop-Harrison et al. 1973), ether extracts will contain lipid material from the entire pollen grain, including the inner wall (intine), cell membrane, and protoplasm, rather than from only the exine surfaces and cavities where the pollenkitt resides. In 1989 we conducted a study to obtain more accurate estimates of the contribution of pollenkitt to pollen weight. We washed pollenkitt from the pollen of four species using the method in Dobson (1988), where hand-collected pollen in 20–40 mg quantities was placed on milipore filters fitted within a syringe and rapidly washed with 0.5 ml hexane in less than 20 seconds. The eluate was collected directly onto filter paper (9 cm diameter), which was allowed to air dry until the solvent had evaporated and was weighed to the nearest 0.05 mg. The findings depicted in Fig. 1 show that for the three insect-pollinated species investigated, pollenkitt represented 3.6–5.5% of the pollen weight, which falls within the common range cited

above for whole-pollen ether extracts. For the single wind-pollinated grass species we examined (Fig. 1), however, we detected no pollenkitt; more refined measurement methods may be required to pick up the smaller amounts of pollenkitt deposited on anemophilous pollen (Hesse 1980).

The suggestion that pollenkitt is the source of pollen odor (Knoll 1930, Porsch 1956, van der Pijl 1964, Faegri and van der Pijl 1979, Zandonella et al. 1981, Buchmann 1983, Bernhardt 1986) was initially substantiated in the diversity of chemicals reported in pollenkitt of a few species (Wittgenstein and Sawicki 1970, Egorov and Egofarova 1971 in Stanley and Linskens 1974, Roberts et al. 1979) and in pollenkitt-containing extracts of pollen (Stanley and Linskens 1974). Subsequently, Dobson (1988) conducted a chemical survey of pollenkitt oils in 69 angiosperm species (mostly insect-pollinated) using thin layer chromatography. She found wide variation between species in terms of the quantity and diversity of neutral lipids, which encompass compounds typically found in plant odors, including isoprenoid compounds, fatty acid derivatives, and benzenoid compounds, as well as mono-, di- and triglycerides, and pigments. Suggestive patterns in the detected compounds followed taxonomic lines, with congeneric species showing strong similarities in their compositions, a finding that has been supported in subsequent analyses of pollenkitt involving a few species (Houston et al. 1993, Pham-Delègue et al. 1994). These studies paved the way for investigations of pollen volatiles (Dobson et al. 1990, 1996; see the section below) and for the confirmation that volatiles contained in pollenkitt are the same as those in pollen headspace.

Evidence that pollenkitt is the site of pollen odor emission was obtained by Dobson et al. (1987), who used the fragrant pollen of *Rosa rugosa* (Rosaceae) as a model for comparing the volatiles in pollen headspace with those in the pollenkitt (washed from pollen with hexane) using gas chromatography. They found that all the main components in the pollen
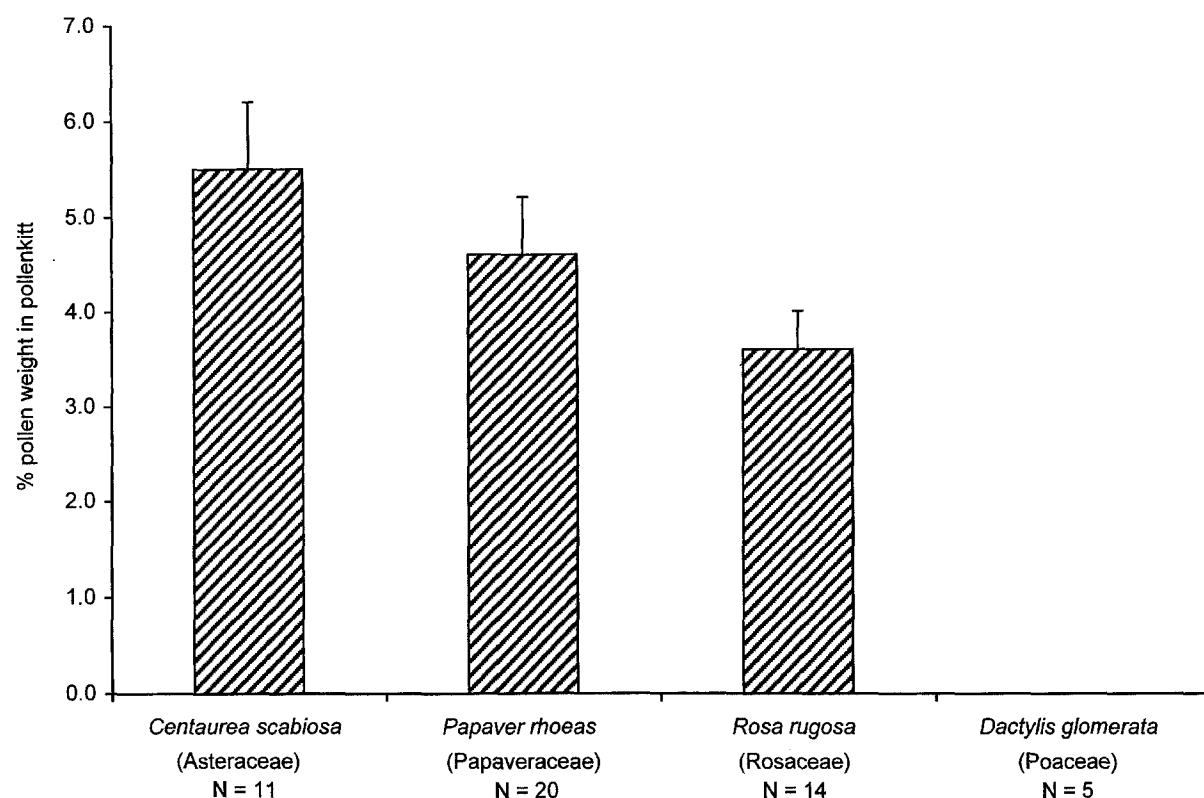
Fig. 1. Percent of pollen weight that is represented by pollenkitt in three entomophilous and one anemophilous species. No pollenkitt was detected in *D. glomerata*. Error bars represent ±1 s.e.m

headspace (Dobson et al. 1987, 1990) were also detected in the pollenkitt (Dobson et al. 1987, Dobson 1989), which confirms that pollen volatiles are indeed coming from the pollenkitt (Table 1). In the analysis, the relative proportions of individual volatiles were often different between the headspace and pollenkitt-hexane samples (Dobson 1989), but this is expected since the headspace shows only the compounds released in the gaseous phase at the time of collection whereas pollenkitt extract shows the total volatiles contained. Subsequent studies of pollen odors have focused on the analysis of headspace samples, since they represent the mixtures of volatiles actually emitted into the air.

In their attempt to open the field of pollen odor studies and lay a foundation in the chemical identification of pollen odor compounds (Dobson et al. 1987, 1990, 1996; Bergström et al. 1995), Dobson and collaborators developed methods to collect and ana-

lyze pollen volatiles by adapting headspace techniques used for flowers (Dobson 1991). The study of pollen odor has been especially challenging due to the low rate of volatile release from pollen compared with other scent-producing floral parts. This slow volatilization may be a consequence of the presence of other lipids in pollenkitt, such as mono-, di- and triglycerides, which can retard the emission of volatiles. However, a gradual release of the finite quantity of pollen volatiles, contained in pollenkitt when it is deposited on the pollen grains prior to anther dehiscence, could be of clear adaptive value to the plant by ensuring that pollen continues to produce odor until it is picked up by pollinators. To overcome the limited emission of volatiles, which reduces the success of volatile detection and identification using gas chromatography and mass spectrometry, 50–200 mg quantities of hand-collected pollen are used for headspace sampling, and volatile collection is carried out over

24–48 hours. It is important that special care be taken to minimize contaminations of the sample, which can obscure the detected volatiles. Furthermore, to identify odors coming specifically from pollen, hand-collected pollen is preferred over the more readily obtained bee-collected pollen (Collin et al. 1995), which may contain various pollen contaminants and show alterations in volatile compositions due to added bee secretions and nectar.

Pollen odor chemistry

To date, pollen headspace volatiles have been analysed in over 15 species from 10 families, but in only 12 species, representing 11 genera and 7 families (all entomophilous, see Table 2), have the collected volatiles been of sufficient quantity for most of the compounds to be identified. In only 6 of these species (*Lupinus polyphyllus*, *Papaver rhoeas*, *Ranunculus acris*, *Filipendula vulgaris*, *Rosa rugosa*,

Table 1. Volatiles detected in pollen headspace and pollenkitt hexane-wash of *Rosa rugosa*. For headspace, XX = "major" compounds detected in amounts ≥2%, X = "minor" compounds in amounts <2% of total; for pollenkitt, X = detected

Compound[a]	Pollen[a]	Pollenkitt[b]
Fatty acid derivatives		
Tridecane	X	
Pentadecane	XX	X
Heneicosane	X	
6-Methyl-5-hepten-2-one	X	
2-Undecanone	X	
2-Tridecanone	XX	X
Tetradecanal	XX	X
Hexadecanal	XX	X
Tetradecyl acetate	XX	X
Hexadecyl acetate	XX	X
Isoprenoids		
Geranial	X	X (?)[c]
Geraniol	X	X
Geranyl acetone	XX	X
Citronellyl acetate	XX	X
Neryl acetate	X	X
Geranyl acetate	XX	X
α-Farnesene	XX	X
Sesquiterpene a	X	
Sesquiterpene b	X	
Sesquiterpene c	X	
Sesquiterpene d	X	
Sesquiterpene f	X	
Sesquiterpene g	X	
Benzenoids		
2-Phenyl ethanol	X	X (?)[c]
Methyleugenol	XX	X
Eugenol	XX	X
2-Phenylethyl acetate	X	X

[a] List includes only compounds detected in pollen odor, and their relative amounts, in Dobson et al. 1990
[b] Modified through reevaluation of data in Dobson et al. 1987
[c] Detection questionable

Lycopersicon esculentum) have the pollen odor compositions been confirmed in repeated samplings (Dobson et al. 1987, 1990, 1996; Bergström et al. 1995; Dobson, Bergström, and Pressman, unpublished). The three major classes of compounds reported in floral scents, namely fatty-acid derivatives, isoprenoids, and benzenoids (Knudsen et al. 1993), are represented in pollen odor, but in amounts that vary among species (Table 2). In half the species all three chemical classes are represented, in the other half only two. Identification of volatiles reveals that each species has its specific pollen-volatile profile representing a unique mixture of compounds. In our studies, pollen odors vary in the number of detected volatiles from 5 to over 30. Most pollen odors are clearly dominated by specific volatiles, belonging to one or more chemical classes. The dominant compounds may comprise one or two volatiles (e.g. *Caltha palustris*, *Filipendula vulgaris*, *Lonicera caprifolium*, *Lupinus polyphyllus*, *Papaver rhoeas*, *Ranunculus acris*) or several volatiles (e.g. *Rosa rugosa*). Some pollen types have strong odors that are readily analyzed using headspace techniques (e.g. *Filipendula vulgaris*, *Papaver rhoeas*, *Rosa rugosa*), whereas others have more subtle odors (e.g. *Centaurea scabiosa*, *Cichorium intybus*, *Lonicera caprifolium*) that can be problematical in distinguishing their components from background noise and establishing their chemical identities. In summary, it can be concluded from these findings that pollen of each species emits its own characteristic mixture of volatiles and that there is no evidence for a general "pollen odor" common to different species.

Included in the analyses above of pollen odor is one species (*Lycopersicon esculentum*) that is buzz-pollinated, where the pollen remains inside the anther until it is released by the vibratory behavior of pollinating bees. Based on subjective evaluations of several buzz-pollinated species with povicidally dehiscent anthers, floral odors appear to arise mainly or exclusively from the anthers (Coleman and Coleman 1982, Buchmann 1983, D'Arcy et al. 1990), and the pollen is described as typically

dry and devoid of pollenkitt. However, SEM studies challenge this view by showing that in *Lycopersicon* (Solanaceae) the pollen is joined by tapetal fluid within the anthers and that in *Actinidia deliciosa* (Actinidiaceae) the amount of pollenkitt varies with anther age (King and Ferguson 1994), suggesting that pollen odor might vary in a similar temporal fashion. Accordingly, in young dehiscing anthers of *A. deliciosa* that contain abundant tapetal material, the pollen is covered with sticky pollenkitt and is probably more scented than in older anthers, where the tapetal material dries, yielding dry pollen with little pollenkitt. The emission of odors from pollen in these plants is supported by our detection of clear pollen odors in *L. esculentum* (Dobson, Bergström, and Pressman, unpublished) and by the behavior of pollinating bees on *A. deliciosa*, which, prior to landing, can recognize the individual flowers (male or female) that have dehiscing anthers (Goodwin and Steven 1993).

Comparative studies of odors from different floral parts indicate that the androecium contrasts with the rest of the flower (Dobson et al. 1990, Knudsen and Tollsten 1991, Sazima et al. 1993, Pichersky et al. 1994, Bergström et al. 1995), thereby showing parallels with visual patterns. The same conclusion is made for pollen as well. Pollen odor has been found to be chemically distinct from the whole-flower odor in all five species (included in Table 2) for which such comparisons have been made. Four of these species offer pollen as the only reward (Dobson et al. 1987, 1990, 1996) and one is nectariferous (Bergström et al. 1995). Differences between pollen and whole-flower odors are apparent in terms of three parameters (Table 3): (1) the total number of volatiles detected in each odor, (2) the number of volatiles that are common to both odors, and (3) the proportional representations in each odor of these volatiles in common. Based on this, contrasts in the odors range from subtle to strong. *Papaver rhoeas* showed the least contrasts, where the pollen and whole flowers differed mainly in terms of the proportional representation of certain major volatiles;

Table 2. General classes of volatiles detected in pollen headspace odors of all species for which compounds have been identified. The chemical classes are rated by their relative representation in each odor, where X = <10%, XX = ≥10, <25%, and XXX = ≥25% of total volatiles. The chemical classification follows that in Knudsen et al. (1993)

Compound	Asteraceae			Caprifoliaceae	Fabaceae	Papaveraceae	Ranunculaceae		Rosaceae			Solanaceae
	Cichorium intybus[a]	Centaurea scabiosa[a]	Sonchus arvensis[a]	Lonicera caprifolium[a]	Lupinus polyphyllus[b]	Papaver rhoeas[b]	Caltha palustris[a]	Ranunculus acris[c]	Filipendula vulgaris[b]	Rosa canina[d]	Rosa rugosa[d,e]	Lycopersicon esculentum[f]
Fatty acid derivatives												
hydrocarbons	XXX	XXX	XXX			XXX			XX	X	X	XXX
aldehydes						X				XXX	XX	X
alcohols					XX		XXX		XXX		XX	
ketones											XX	
esters												
lactones								XXX				
Isoprenoids												
monoterpenes	X	XX	XX		XX	X					XX	X
sesquiterpenes	X	X	X		XXX	X	X	X			X	X
irregular terpenes				XXX	XX	X				XX	X	XX
Benzenoids												
aldehydes	X	X	X		X	X			X			
alcohols	X	XX	XX	XX				XX	XX		XX	
ketones												
esters											X	

[a] Dobson, Bergström, and Groth, unpublished: incomplete data or based on one sample only
[b] Dobson et al. 1996
[c] Bergström et al. 1995
[d] Dobson et al. 1987
[e] Dobson et al. 1990
[f] Dobson, Bergström, and Pressman, unpublished

Table 3. Comparison between pollen and whole-flower odors in terms of total number of volatiles detected, number of volatiles common to both, and differences in the percent representation of volatiles in common, where X = weakly, XX = moderately, and XXX = strongly different. Data are shown for all five species investigated to date

| Species	Total number of volatiles		Number of volatiles in common	Differences in % representation
	Whole flowers	Pollen		
Fabaceae				
Lupinus polyphyllus[a]	37	24	22	XX
Papaveraceae				
Papaver rhoeas[a]	26	33	22	X
Ranunculaceae				
Ranunculus acris[b]	30	5	5	XXX
Rosaceae				
Filipendula vulgaris[a]	20	10	10	XXX
Rosa rugosa[c]	24	29	17	XXX

[a] Dobson et al. 1996
[b] Bergström et al. 1995
[c] Dobson et al. 1990

curiously, one third of the pollen volatiles were not detected in whole-flowers, but all were minor components in pollen odor and were probably at levels too low to be detected in the whole-flower odors. Contrasts were moderate in *Lupinus polyphyllus* in all three aspects. In both *Ranunculus acris* and *Filipendula vulgaris*, the contrasts were strong: pollen odor had markedly fewer volatiles, of which all were detected in whole-flowers but in often strikingly different proportions, with dominant compounds in pollen being only minor components of the whole-flower odor (and dominant volatiles in whole flowers being conversely often not detected in pollen odor). The most complex contrast, however, was presented by *Rosa rugosa*, which has a pollen odor composed of a similar number of volatiles as in whole flowers but where many (including major) volatiles are of distinct identity, and where volatiles in common were present in markedly different proportions.

The evidence is clear that pollen has an odor upon its exposure from the dehiscing anther, because it is already covered with pollenkitt. The pollenkitt substances are formed inside the tapetal cells which, prior to anther dehiscence, break down and empty their contents into the anther loculus, where pollenkitt fills the exine cavities and covers the outer surface of the pollen grains (Pacini et al. 1985, Hesse and Hess 1993). Thus, pollenkitt – and pollen odor – is of sporophytic origin. However, the question remains as to what extent pollen odor can be modified through the uptake by pollenkitt of volatiles released from other flower parts during the time interval between anther dehiscence and pollen removal by pollinators. In *Rosa rugosa*, the volatile profile of pollen is strikingly different from that of the petals, but when pollen is left on the intact flower for over four hours after the anthers dehisce, greater quantities of mainly petal-originating volatiles may be found in the pollen odors than when the petals are removed upon anthesis. This suggests that the pollenkitt adsorbed the volatiles from the surrounding air laden with mainly petal odors (Dobson et al. 1990, Fig. 2). This effect could be of varying importance depending on the plant species and the time its pollen remains in the open anther before being removed.

Rosa rugosa flowers

Rosa rugosa pollen

Centaurea scabiosa pollen

Papaver rhoeas pollen
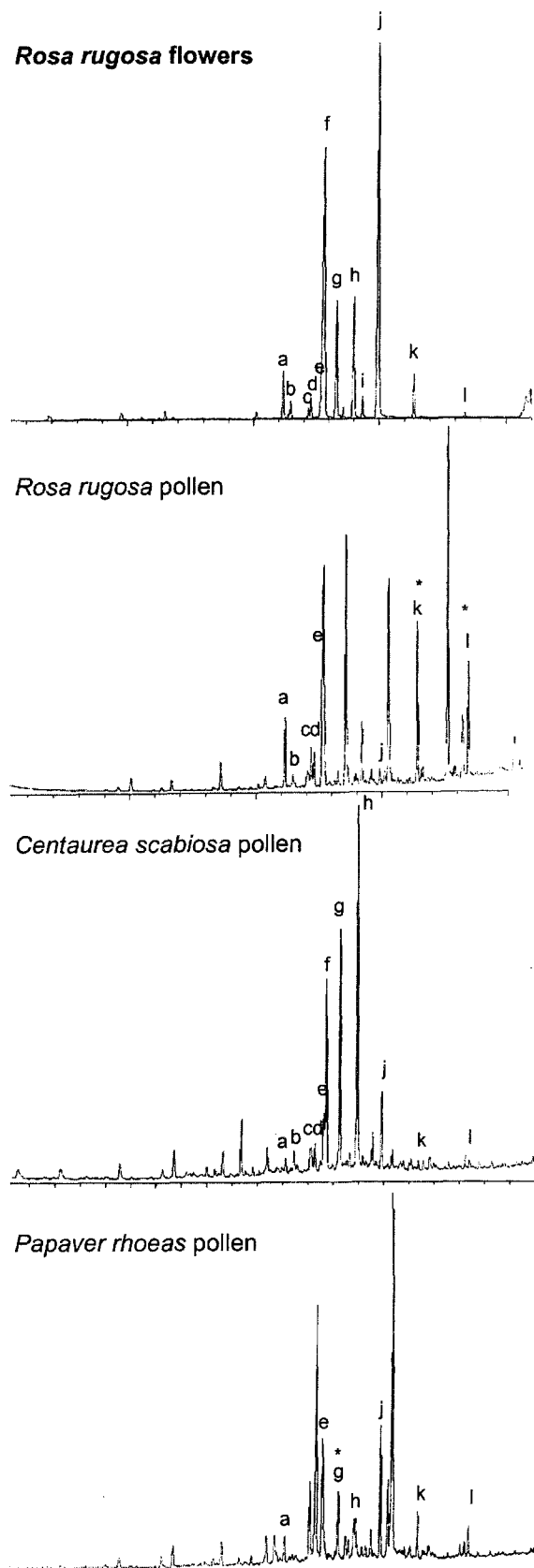
To evaluate how uptake of environmental volatiles by pollen can differ among species, we conducted a study in 1988 where we exposed pollen of two other species to the floral volatiles of Rosa rugosa. We placed 100–130 mg freshly collected pollen in a Petri dish (10 cm diameter) lined with aluminum foil and surrounded it by a tight circle (15 cm diameter) of 20 newly opened intact R. rugosa flowers, elevating the pollen dish to situate it at the level of the petals. Pollen samples were left among the flowers for six hours and headspace volatiles were collected from the pollen using methods in Dobson (1991). As controls, we collected headspace odors from freshly-collected pollen of each species. R. rugosa flowers have an odor profile dominated principally by petal-originating monoterpenoid alcohols and 2-phenyl ethanol, and to a lesser degree by the androecium-originating volatiles eugenol and methyleugenol; trans-α-farnesene is a minor component (Dobson et al. 1990), as shown in Fig. 2. The adsorption of airborne R. rugosa volatiles onto pollen varied among the species, but certain compounds were adsorbed consistently by all three pollen types tested, namely 2-phenyl ethanol and trans-α-farnesene (Fig. 2). When R. rugosa pollen, which has a strong odor that overlaps little in chemical composition with that of the petals, was exposed to the flowers, its volatile profile was modified by the adsorption of small quantities of some petal volatiles, but this uptake was

Fig. 2. Gas chromatograms of pollen odors following pollen exposure to intact flowers of Rosa rugosa for 6 hours. R. rugosa pollen was exposed by leaving pollen on flowers; C. scabiosa and P. rhoeas pollen was exposed by placing pollen in dishes surrounded by R. rugosa flowers. Lettered peaks indicate only volatiles present in odors of R. rugosa flowers, with an asterisk marking those also present in the pollen's own odor: a citronellyl acetate, b neral, c neryl acetate, d geranial, e α-farnesene, f citronellol, g nerol, h geraniol, i phenyl methanol, j 2-phenyl ethanol, k methyl eugenol, l eugenol. All other peaks correspond to volatiles detected exclusively in pollen; for identities, see Dobson et al. 1990, 1996

selective, since the three dominant monoterpene alcohols citronellol, nerol, and geraniol, were not detected. Pollen of *Centaurea scabiosa* (Asteraceae), which has a weak odor but is very sticky and seems to have abundant pollenkitt, changed markedly in its odor after exposure to the flowers. The prominent monoterpenoid alcohols (citronellol, geraniol, and nerol) were taken up the most, followed by *trans*-α-farnesene and 2-phenyl ethanol; several other compounds were adsorbed in minor quantities. Finally, pollen of *Papaver rhoeas*, which has a pronounced odor dominated by long-chain (C_{17} and C_{19}) hydrocarbons, showed at most only a moderate uptake of *trans*-α-farnesene, 2-phenyl ethanol, and possibly nerol (which is also a component of its own odor), and minor uptake of geraniol, methyleugenol, and citronellyl acetate. One trial was also made with pollen of the wind-pollinated *Dactylis glomerata* (Poaceae), but the odor sample collected was very weak and no volatiles could be conclusively identified; the low uptake could well be a reflection of the small quantities of pollenkitt on its pollen grains.

From our findings of the uptake of environmental volatiles by pollenkitt, we can conclude that: (1) pollen species vary in the amount and kind of volatiles adsorbed from the surrounding air, and this seems to be influenced by both the amount and chemistry of the pollenkitt; (2) volatile adsorption is selective, where certain volatiles are taken up more consistently across species than others and the amount of each volatile adsorbed is not necessarily correlated with its relative abundance in the air.

Pollen odor in relation to mode of pollination

If a primary function of pollen volatiles is to enhance pollination by pollen-foraging insects, one would expect flowers pollinated by nectar-seeking animals or by wind to have less prominent and distinctive pollen odors. Knoll (1930) pointed to pollen odor as playing an important role in attracting pollinator insects,

based on the observation that one finds very strong smelling pollen in various insect flowers in contrast to typical wind flowers. Strong pollen odors have in fact been described mainly in plants pollinated by pollen-feeding insects, especially beetles (Porsch 1956), such as *Lithocarpus densiflorus* (Fagaceae) and Cycadaceae (Faegri and van der Pijl 1979). However, Porsch (1956) argued that since pollen of wind-pollinated plants is equipped with pollenkitt, it too must have characteristic odors. He pointed out that several wind-pollinated species have prominent pollen odors (e.g. species in Cyperaceae) and that interestingly, most of these are also visited by pollen-feeding insects. Porsch provided numerous examples of beetles, as well as flies and bees, observed feeding on pollen from plants typically considered to be wind-pollinated, suggesting that pollen odor, alone or together with color, attracts the insects.

To determine if pollen odor is correlated with pollination mode, and the need to attract pollinators, von Aufsess (1960) compared the odor of pollen from plants depending on wind, insects, or birds for pollination using both subjective human evaluation and discrimination by honey bees in learning trials. In subjective evaluations, pollen of 13 anemophilous species (mainly Poaceae) had weaker odors that were also of a completely different quality compared to entomophilous pollen, suggesting that there are major differences in the types of volatiles present. However, anther scents in anemophilous plants were most often stronger and qualitatively different from inflorescence odors, but it is not clear how much of this contrast is due to pollen. In our studies of pollen headspace we examined four anemophilous species, *Pinus sylvestris*, *Betula verrucosa*, *Quercus robur*, and *Dactylis glomerata*, but in all cases the volatile profiles were very weak and further study is needed to identify the compounds.

Furthermore, headspace odors from anemophilous flowers suggest that male flowers may have greater proportions of α-methyl ketones and alcohols than either the corresponding

female flowers or animal-pollinated species. In our study of *Rumex acetosa* (Polygonaceae), which is dioecious, volatile profiles of the male and female flowers were similar except for the exclusive detection of α-methyl ketones and alcohols in the male ones (Table 4). A similar trend is apparent in the wind-pollinated *Cycas rumphii* (Cycadaceae) (Pellmyr et al. 1991), where male-cone odors displayed a greater preponderance of α-methyl ketones and alcohols than female-cone odors, which had more aliphatic esters (Table 4). As a point of emphasis, no α-methyl ketones or alcohols were detected in the odors of male cones of four simultaneously studied insect-pollinated cycads (Pellmyr et al. 1991). While these findings are difficult to extrapolate to pollen, since it is not known what contribution pollen is making to these whole-flower or cone odors, our studies of pollen headspace in entomophilous species lend some support to the proposed connection between α-methyl ketones and anemophily, or at least decreased entomophily. Indeed, in *Filipendula vulgaris* the pollen odor is strongly dominated by 2-heptadecanone, and the flowers, which attract only few insects, have been

suggested to be partly or mainly wind-pollinated (see Dobson et al. 1996). We have also detected α-methyl ketones in pollen odor of the bee-pollinated *Rosa rugosa*, which has a complex pollen odor that includes among its major components two α-methyl ketones (Dobson et al. 1990), of which one, and possibly both, deter landing by pollinating bumble bees when tested alone (Dobson et al. 1999). However, the pollen odor is also dominated by volatiles strongly attractive to bumble bees (e.g. eugenol), which appear to overshadow the deterrent effect of the α-methyl ketones. Taken together, these findings tentatively suggest that a major presence of α-methyl ketones and alcohols in flower and pollen odors may be associated with anemophily, or with reduced entomophily if these compounds are not counteracted by the presence of volatiles that are attractive to insects.

Comparative studies of pollen odor in plants pollinated by different animal groups are few, but they reveal patterns that are associated with the occurrence of pollen-feeding. Based on subjective human evaluations, species relying on pollen-feeding insects had

Table 4. Percent representation of different compounds in headspace volatiles from female and male flowers or cones of two wind-pollinated plants

Compound	*Rumex acetosa* (Polygonaceae)		*Cycas rumphii*[a] (Cycadaceae)	
	Female	Male	Female	Male
Fatty acid derivatives				
"green-leaf" (*cis*-3-hexenyl acetate, *cis*-3-hexenol, hexanol)	21.3	1.2	0.2	0.5
α-methyl ketones	–	**19.2**	**4.2**	**15.8**
α-methyl alcohols	–	**14.4**	**25.6**	**51.4**
esters			69.4	32.1
Isoprenoids				
monoterpenes	28.1	15.8	0.5	–
sesquiterpenes	47.2	46.8		
irregular terpenes (6-methyl-5-hepten-2-one)	1.8	1.1		
Benzenoids				
alcohols and esters	1.5	1.4		

[a] Pellmyr et al. 1991

stronger pollen odors than did bird-pollinated plants in 22 of 24 pair-wise subjective comparisons (von Aufsess 1960). In the pollenkitt survey by Dobson (1988), the few species that are pollinated by animals seeking rewards other than pollen (i.e. Lepidoptera and hummingbirds) tended to have a pollenkitt with relatively few constituents compared to bee-pollinated species. Furthermore, human evaluations showed that pollen odor in entomophilous plants was qualitatively different from the petal odor in 15 of 17 species and in most cases was stronger, whereas in bird-pollinated plants it was qualitatively different in only 4 of 20 species and was equally often stronger as weaker. This more pronounced olfactory contrast of pollen against the remaining flower in entomophilous than in ornithophilous species was supported in bee training experiments. Honey bees clearly distinguished between the stamens and flowers of *Oenothera fruticosa* and between the pollen and flowers of a rose hybrid, but showed little or no ability to discriminate stamens against flowers in 5 of 6 ornithophilous species. This suggests that pollen scent in bird-pollinated plants is either too weak or is not sufficiently different from the rest of the flower for detection by bees.

While there appears to be a general trend for pollen odor to differ in association with pollination mode, the evidence is spotty and research is needed to get a more definitive picture of how pollen-vector type may have influenced the evolutionary selection for pollen odor emission. Comparative chemical studies of pollen odor between plants differing in pollination mode and of odor patterns in flowers presenting special cases of insect pollination, would help clarify to what extent pollen odor has evolved in association with the need to attract pollen-foraging pollinators. Some examples of insect-pollinated species that would be of special interest for investigation include those that have heterantherous flowers with fodder and fertilizing pollen (Endress 1994b), where fodder pollen might be olfactorily more attractive than fertilizing

pollen; nectarless species with rewardless female flowers that appear to mimic male flowers possibly through odor (Ågren and Schemske 1991); and cryptically dioecious species with poricidal anther dehiscence, where female flowers producing non-functional pollen are visited by pollen-foraging bees, suggesting that pollen odor is similar in both male and female flowers (Levine and Anderson 1986, Cane 1993, Knapp et al. 1998).

Effects of pollen odors on animals

Pollen is an important food (especially protein) source for many flower-feeding insects, but studies investigating how pollen stimuli, mainly visual and olfactory, influence the location and selection of pollen by insects are few (Dobson 1994). The distance at which pollen odors operate undoubtedly depends on both plant and insect species involved, but given that pollen odors are generally quantitatively weak compared to those of the whole flower, they are probably perceived by insects mainly at short distances, just prior to and after alighting on the flower. Furthermore, individual volatiles in the odor may be perceived at different distances within this close range, as determined by their volatility. At pre-alighting distances, pollen odors could allow insects both to recognize different pollen host-plants and to assess the amount of pollen reward, whereas after alighting they could help guide insects to the pollen and provide further species-specific recognition cues, including some that stimulate feeding. At any stage of plant selection, other stimuli (visual, tactile, gustatory) may act in concert with olfactory cues, with the relative importance of each depending on the particular insect-flower interaction in question (Dobson 1994). In honey bees and bumble bees, odors are learned more rapidly and with a greater retention than colors, and evoke stronger discrimination between flowers (e.g. von Frisch 1923, Kugler 1943, Menzel 1985, and see Dobson 1994), which places them higher in the hierarchy of floral stimuli that modulate flower and pollen selection.

By having pollen with a distinct odor, a plant gains both an additional species-specific identification label by which bees and other flower-visiting insects can discriminate it from other species and a means of advertising the presence of pollen rewards. The pollination-related advantages conferred on a plant from having pollen odor could translate into both greater foraging efficiency for the insect, through more efficient location and selection of food plants, as well as in increased fitness (especially male fitness) for the plant, through enhanced flower constancy, greater pollen export, and more effective pollen transport to stigmas.

Volatiles from pollen have been implicated in the host-plant selection of several pollen-feeding insects, with most evidence coming from studies of bees. Among non-bee insects, pollen odors appear to be attractants to plant-specialist beetles, including the weevil *Bruchus pisorum* (Bruchidae) which feeds on *Pisum* (Fabaceae) pollen (Stephen L. Clement, pers. comm. 1993), *Zygogramma bicolorata* (Chrysomelidae) which feeds on pollen and other plant parts of *Parthenium hysterophorus* (Asteraceae) (Jayanth et al. 1993), and the pollen beetle, *Meligethes aeneus* (Nitidulidae), which feeds on oilseed rape pollen during both adult and larval stages (Charpentier 1985). Recent olfactometer studies show that adult *M. aeneus* are attracted to flowers by odors, including those from pollen, and that they prefer male-fertile over male-sterile plants both in their general attraction to plants during bloom and in their oviposition, which implies the involvement of pollen odor (Samantha Cook, pers. comm. 1999). A plant-specific thrips species appears to use pollen odor to recognize its host-plant pollen, whereby it inspects pollen grains without touching them prior to initiating any probing (Kirk 1985). In the sunflower moth *Homoeosoma electellum* (Pyralidae), which requires sunflower pollen during the larval stage, pollen volatiles physiologically affect virgin females by triggering them to initiate calling behavior earlier, to spend more time calling, and to have a higher

rate of egg maturation (McNeil and Delisle 1989), which allows the females to adjust their reproduction to the temporally and spatially variable availability of pollen. In addition, pollen odor contains a volatile oviposition stimulant that enhances the female's location of newly opened sunflower heads (Delisle et al. 1989). At least some of the same volatiles might be involved in the initiation of calling behavior and oviposition by the European sunflower moth *H. nebulellum*, where both behaviors are induced in part by pollen volatiles detected at a distance ≥3 cm (Le Metayer et al. 1993). A greater effect of volatiles might have been obtained had the distance to the odor source (i.e. pollen test sample) been shortened, which would have increased the moth's perception of any active chemicals with low volatility.

Bees clearly perceive pollen odors. Pollen volatiles may be required to elicit landing, as in foraging-naive bumble bees, where it is most effectively elicited by a combination of visual signals from the anthers and olfactory stimuli from the pollen (entomophilous only) (Lunau 1992). Kleptoparasitic *Nomada* (Anthophoridae) bees are more attracted to host nests containing pollen than to those without (Cane 1983). When offered a choice of pollen from different species, honey bees inspect each pollen dish by hovering above it, implicating that they are using odor and/or color to evaluate the pollen prior to landing (Schmidt 1982). Both von Frisch (1923) and von Aufsess (1960) were able to train honey bees to differentiate between odors of pollen and other floral parts, indicating that the distinctive odors of pollen within the whole-flower context are perceived by bees, even in flowers where the contrasts are subtle (e.g. *Papaver*).

Honey bees are olfactorily attracted to pollen (Doull and Standifer 1969, 1970, Doull 1974a, b) and can use pollen odor to discriminate between plant species when these are offered as either whole pollen (Levin and Bohart 1955, Doull and Standifer 1969) or pollen extracts (Doull and Standifer 1970), based on the different numbers of attracted

bees. Ether extracts of pollen, which contain pollenkitt and thus pollen odor compounds, are attractive to foraging honey bees and stimulate them to collect non-pollen materials that are otherwise avoided (Louveaux 1959, Hügel 1962, Taber 1963, Lepage and Boch 1968, Hohmann 1970). Honey bees can be trained to olfactorily discriminate between pollen of different species (von Frisch 1923, von Aufsess 1960, Samantha Cook, pers. comm. 1999), and specific odors carried on bees returning to the hive play a central role in the recruitment of foragers to new pollen sources (von Frisch 1923). Pollen selection may be mediated in part by specific volatiles, given that chemicals attractive to honey bees, including unidentified sterol-related compounds (Louveaux 1959, Hügel 1962) a carotenoid ester and a free fatty acid (Standifer 1966, Lepage and Boch 1968, Hopkins et al. 1969), and to pollen-specialist solitary bees (Dobson, unpubl.), have been isolated from pollen. Deterrent compounds might also influence pollen selection (Free 1970, Schmidt 1982), as suggested especially when bees actively avoid pollen or remove from their bodies any pollen incidentally picked up from flowers (Cazier and Linsley 1974, Hurd et al. 1980, Waller et al. 1984).

Pollen odor can present a more species-specific chemical signal than the whole-flower fragrance by including volatiles that are not commonly emitted by nonpollen floral parts or that are only minor constituents in whole-flower odors. This is exemplified in the high representation of particular defensive chemicals, such as the lactone protoanemonin in pollen odor of Ranunculus acris (Bergström et al. 1995), or of various compounds including α-methyl ketones in Rosa rugosa pollen (Dobson et al. 1990, 1999). Conversely, pollen odor can become a more species-specific signal through the absence of major volatiles emitted from other flower parts, which tend to be more common in flower fragrances in general, as occurs in both the aforementioned species and in Lupinus polyphyllus (Dobson et al. 1996).

Behavioral studies with pollen-specialist solitary bee species indicate that the species-specificity of pollen odor can be of key importance in pollen host-plant recognition. Dobson and her students have tested four pollen-specialist bee species to determine what floral stimuli bees use to initially recognize their host-plants and how a bee's search image is modified after foraging experience. They used multiple-choice experiments, where bees were offered a choice of different plant species in the form of floral or pollen odors, and where bee preferences were measured by their feeding-attempt responses. When offered colors only, all bee species showed an innate feeding preference for yellow; the yellow stimulus was also required for three species to exhibit responses to odors. When tested with odors, preference patterns varied among the species (Table 5). In the most narrowly specialist bee, Chelostoma florisomne, which restricts its pollen and generally nectar foraging to buttercups (Ranunculus spp.), foraging-naive females could recognize their host plant more effectively when offered pollen odors than floral odors, and subsequent studies found that pollen odor contains a key chemical used in flower recognition (Dobson, unpubl.). However, after gaining foraging experience in the field, bees relied more on floral odors, which operate at longer distances than pollen odors (≤1 cm was needed for responses to pollen volatiles). In a population of Colletes fulgidus that specializes with respect to pollen foraging but is more generalist in its nectar feeding, foraging-naive females could recognize their pollen host-plant when offered either floral or pollen odors, both of which have strong and quite similar scents to the human nose; following foraging experience, this bee also appears to depend more heavily on floral stimuli (Dobson 1987). The other two bee species studied, Dasypoda hirtipes and Heriades truncorum, are more broadly specialized at the plant tribe or family level and have proved to be more challenging in the attempts to determine what role pollen odor plays in their host-plant recognition. Indeed, prefer-

Table 5. Host-plant recognition by pollen-specialist bees when offered a simultaneous choice of flower or pollen odors from four species. Recognition is based on the display of significantly more feeding attempts towards pollen host-plant(s) than nonhost-plants. Parentheses indicate incomplete data sets; (–) = not tested. All data are unpublished unless indicated

Bee species	Flower odors	Pollen odors
1. Chelostoma florisomne (Megachilidae) host plants: *Ranunculus* spp (Ranunculaceae)		
1) foraging-naïve	no	yes
2) foraging-experienced	yes	no
2. Colletes fulgidus longiplumosus (Colletidae)[a] host plants: *Grindelia stricta* (Asteraceae)		
1) foraging-naïve	yes	yes
2) foraging-experienced	(yes/no)	(no)
3. Dasypoda hirtipes (Melittidae) host plants: Asteraceae: Cichorioideae		
1) foraging-naïve	(no)	(no)
2) foraging-experienced	no	(–)
4. Heriades truncorum (Megachilidae) host plants: Asteraceae		
1) foraging-naïve	yes/no	yes/no
2) foraging-experienced	(–)	(–)

[a] Dobson 1987

ences by these bees to either flower or pollen odors have being equivocal or not apparent (Dobson, unpubl.). These comparative studies across bee species make it clear that floral odor parameters characterizing host-plant selection in one bee-flower association do not necessarily apply to others; each association must be examined within its own biological and ecological context.

The presence in pollen of unusual volatiles, possibly with defensive functions, may be an essential feature that allows pollen-feeding insects to become specialized on particular plants, where the species- or genus-specific volatiles can serve as key recognition stimuli in host-plant location and selection. This scenario is evident in the specialization of the solitary bee *Chelostoma florisomne* on pollen of *Ranunculus*, where newly-emerged bees rely on pollen odor, and more specifically on its major component protoanemonin, to recognize their host plants (Dobson, unpubl.). A similar situation is suggested for the beetle

Zygogramma bicolorata (Chrysomelidae) that is a specialist on *Parthenium hysterophorus* (Asteraceae), where the sesquiterpene lactone parthenin attracts the beetles and induces them to feed (Jayanth et al. 1993). While the exact location of parthenin in the pollen has not been identified, the beetle's response is elicited when parthenin is presented either as whole pollen or as an aqueous extract of pollen, which is obtained in a process that would also wash off the pollenkitt; this suggests that some parthenin is contained in the pollenkitt, where it could olfactorily attract the beetles.

Pollenkitt can contain diverse compounds covering a range of polarities (Dobson 1988), and pollen compounds recently identified as feeding stimulants might possibly reside in part in the pollenkitt. Phagostimulant chemicals in pollen have been implicated in feeding preferences (based on consumption) by honey bees for one pollen over another (Schmidt and Johnson 1984) and by the hover fly *Eristalis tenax* (Syrphidae) for whole pollen or pollen

extracts over extracted pollen (Wacht et al. 1996). A variety of compounds appear to be involved, given that pollen extracts of different polarities stimulate feeding in honey bees (Robinson and Nation 1968, Schmidt 1985) and in the western corn rootworm beetle, *Diabrotica virgifera virgifera* (Chrysomelidae) (Hollister and Mullin 1999). Feeding stimulants in the hoverfly consist mainly of amino acids (Wacht et al. 2000), which are contained in pollen parts other than pollenkitt. However, chemicals in sunflower pollen that are strongly phagostimulatory to the western corn rootworm are more diverse, comprising lipid and midpolarity compounds that include primary common nutrients (fatty acids, glycerides, amino acids) as well as secondary chemicals, such as phenolic polyamides and flavonols (Lin and Mullin 1999). Some of these probably occur in pollenkitt, and some might be of sufficient volatility to act as very close-range olfactory stimuli. When dealing with pollen odor compounds of low volatility, it can be difficult to distinguish whether they are perceived through the modalities of olfaction or gustation, and behavioral data must be interpreted with care. Host-plant discrimination by pollen-feeding insects, including the two Asteraceae-pollen specialist bees discussed above, might likewise involve very short-range pollen volatiles that are in quantities too small to be perceived at the distances used in behavioral experiments testing for insect responses to pollen odors (e.g. 1 cm), and that may consequently be detected only in feeding tests.

Through the distinctness of pollen odor against the whole flower, pollen volatiles could provide pollinators a means for assessing pollen availability, thus allowing the insect to forage more efficiently by restricting its visits to the most rewarding flowers. The use of pollen odor to locate flowers offering pollen is suggested in observations of solitary bees cruising or gnawing nearly open flower buds that contain already dehiscing anthers (Hurd and Linsley 1963, Linsley et al. 1963, Eickwort 1973). Its further use to quantify a flower's pollen rewards is implicated in honey bees

preferentially visiting flowers of *Papaver* with the greatest quantity of pollen (Ribbands 1949). Foraging bees are often reported to "inspect" flowers, where they briefly hover above a flower prior to either landing or flying on (e.g. Ribbands 1949, McNaughton and Harper 1960, Heinrich 1979, Zimmerman 1982, Dobson et al. 1999). These inspections could be crucial times when bees evaluate close-range cues, both visual and olfactory, that allow them to assess pollen availability. This is supported by the increase in inspection frequency with time of day, as food rewards become depleted and bees show more selectivity in the flowers they visit. In flowers where pollen is fully exposed (Ribbands 1949, Zimmerman 1982, Cresswell and Robertson 1994) or where pollen depletion is accompanied by obvious visual cues, including age-related changes in floral morphology (Pellmyr 1988), post-pollination color changes (Wainright 1978), or visible alterations in anthers (Zimmerman 1982), it is difficult to establish the extent to which bees are processing visual as opposed to olfactory cues during an inspection. Pollen odor cues are, however, strongly implicated in some flowers that have concealed pollen, where bees have been observed assessing rewards prior to selecting individual flowers with the most pollen whether or not flowers display visual cues associated with changes in pollen availability (Heinrich 1979, Haynes and Mesler 1984, Gori 1989, Harder 1990, Shelly et al. 1993). Allowing pollinators to assess pollen rewards might be a primary selective force driving the evolution of distinctive pollen odor in some species (e.g. *Lupinus polyphyllus*, see Dobson et al. 1996). Pollen odor as a means of assessing food reward may also explain the preference of the syrphid fly *Episyrphus balteatus* for oil-seed rape flowers in which the number of anthers was experimentally increased (Golding et al. 1999).

The strongest evidence for the use of pollen odor by bees for discriminating between flowers that have different amounts of pollen comes from behavioral field studies of pollen-foraging bumble bees on *Rosa rugosa* (Dobson

et al. 1999). By variously altering the androecia of flowers presented to free-flying bees on blooming bushes, it is clear that bumble bees rely more heavily on cues (both visual and olfactory) from the androecium than from the petals to distinguish between pollen-offering first-day flowers and pollenless second-day flowers, and furthermore, that pollen odor modulates landing and pollen foraging on first-day flowers. Indeed, addition of certain major pollen volatiles, namely eugenol and tetradecyl acetate, to flowers from which anthers had been removed significantly increased both landings and typical vibratory pollen-collecting behavior in bumble bees. Interestingly, not all components of pollen odor enhanced flower visitation, with the α-methyl ketone 2-tridecanone and geranyl acetate having slightly deterrent effects on landing frequency. While pollinators encounter the entire mixture of volatiles in a pollen odor, their responses appear to be governed by their differential perception of the individual chemicals and by any interactive effects, both additive and synergistic, among the volatile constituents. The findings that volatiles in pollen odor can include chemicals that have differing effects, both attractive and deterrent, on pollinators point to pollen volatiles as playing multiple roles in the biology of pollen.

Pollen odors in plant defense

The apparently ubiquitous occurrence of odor in pollen, its varying chemical composition, and its inclusion of insect repellents and antimicrobial compounds, suggest that pollen odor chemicals serve other purposes in addition to pollinator attraction. These other functions include, most importantly, the widespread need to defend the male gametophyte against destructive pollen-feeding animals and pathogens, as well as the occasional defense of the plant against competition from other species through the phenomenon of pollen allelopathy.

Volatiles that deter non-pollinator insects from feeding on various floral parts, including

pollen, may be located in different parts of the flower (Casida 1980, Mullin et al. 1991, Wells et al. 1993). The presence of some of these chemicals in non-pollen floral parts might reduce the need for plants to deposit deterrent chemicals in the pollen, including the pollen-kitt. Anthers may keep pollen-feeding insects at bay by containing repellent chemicals (Belcher et al. 1983, Rossiter et al. 1986, Fujimori and Ashihara 1993, Werner et al. 1995). Alternatively, attractive volatiles in sterile pollen of heterantherous flowers (Faden 1992) or in highly attractive food structures, such as staminodes (Bergström et al. 1991, Endress 1994b) may lure pollen-feeders away from the fertile pollen. For plants with documented defensive compounds in anthers, it would be instructive to extend chemical analyses to pollen to clarify whether the defense role is relegated to anther tissue rather than pollen, or whether pollen also contains some of the same defenses, which might compromise its attraction to pollinators. It seems probable that plants with exposed pollen that is readily exploited by non-pollinating insects would be under greater selection pressure to produce antiherbivore deterrents in pollen, as found in *Rosa rugosa* (see below, Dobson et al. 1990) and *Ranunculus acris* (with protoanemonin) (Bergström et al. 1995).

The α-methyl ketones detected in pollen odors (discussed in sections above) appear to serve in defense against both insects and pathogens. The two abundant α-methyl ketones in pollen odor of *Rosa rugosa*, namely 2-undecanone and 2-tridecanone (Dobson et al. 1990), are deterrent and even toxic to several insects (see Kennedy et al. 1991, Farrar et al. 1992, Marr and Tang 1992, Maluf et al. 1997), and some α-methyl ketones show antifungal activity (Cole et al. 1975). In *R. rugosa*, 2-tridecanone is also a deterrent to pollinating bumble bees (Dobson et al. 1999), suggesting that some defense volatiles are broadly acting. In this particular example, the presence of highly attractive chemicals in the pollen odor (i.e. eugenol, tetradecyl acetate) appears to overshadow the deterrent effects of defense

compounds on bumble bees and allows the flowers to still rely on bees for pollination while the pollen is protected against pathogens and possibly certain herbivores. The α-methyl ketone 2-heptadecanone that strongly dominates the pollen odor of *Filipendula vulgaris* may likewise be one reason why the flowers attract few insects. Wind-pollinated plants have no need to attract pollinator insects, and one would therefore expect defensive chemicals to be more prominent in their pollen odor, which may explain the suggestive higher incidence of α-methyl ketones in anemophilous male flowers as compared to either their female counterparts or entomophilous flowers.

Various other defense compounds have been isolated from pollen, and although many may be constituents of pollen odor, in only few cases has this been experimentally confirmed. Compounds of mid to high polarity in whole pollen or aqueous pollen extracts, but not pollen hexane extracts, from sunflower are involved in the deterrence of ovipositing females of the banded sunflower moth *Cochylis hospes* (Cochylidae), which lay eggs on bracts even though larvae feed on pollen, but it is not clear if pollen volatiles play a role (Barker and Grugel 1996). Hydroxycinnamic acid-spermidine amides, which are located mainly in the pollen exine (and pollenkitt?) and are thought to function in defense against pathogens, have been isolated from genera in several orders by extracting pollen with methanol (Meurer et al. 1988), a process that would also remove the pollenkitt. Sesquiterpene lactones appear to have evolved as defense through their deterrency to herbivores and activity against fungi and bacteria (Picman 1986), and at least one has been isolated in pollen (Sukhada and Jayanchandra 1980a, b), where it might be contained in pollenkitt (see section above). It seems probable that sesquiterpene lactones are more widespread in pollen, considering that they occur in several angiosperm families and represent over half of the diverse sesquiterpenes that characterize all but one tribe of the Asteraceae (Langenheim 1994). Toxicity against fungi has been demonstrated in some pollen (Char and

Bhat 1975, Pandey et al. 1983, Tripathi et al. 1985), but the chemicals responsible for this activity remain to be isolated.

Volatiles in pollen odors may individually play more than one role in pollen biology. A diversity of essential-oil volatiles that include compounds reported in pollen odors and that are generally interpreted to attract pollinators might also serve as defense against bacteria and fungi (e.g. Morris et al. 1979, Knobloch et al. 1989, Kubo et al. 1995). For example, eugenol, a major pollen volatile in *Rosa rugosa*, both attracts a variety of insects and has known antimicrobial activity (Morris et al. 1979, Zaika 1988). From the insect perspective, pollen defense chemicals may become primary attractants to specialist pollinators or other specialist pollen feeders, as discussed in the previous section for the bee *Chelostoma florisomne* (Dobson, unpublished) or beetle *Zygogramma bicolorata* (Jayanth et al. 1993), and it is probable that other examples will be uncovered with more research. In the case of the sesquiterpene lactone parthenin, three different functions appear to be at play, including general defense, attraction of a specialist herbivore, and pollen allelopathy (Jayanth et al. 1993).

A rarely documented role for pollen volatiles is in the phenomenon of pollen allelopathy, where pollen of one species and has a detrimental effect on the pollen (and pollen germination) of other species, and whereby the species with allelopathic activity gains a competitive advantage and can increase in abundance (Murphy 1999). Pollen allelopathy has been demonstrated in three wind-pollinated species (two Poaceae and one Asteraceae) and agamospermous species of *Hieracium* (Asteraceae). Activity has been attributed to terpenoid and phenolic compounds that are extracted with water from pollen. In the grasses they are thought to be held in the intine and synthesized in the pollen cytoplasm (see Murphy 1999). In *Parthenium hysterophorus* (Asteraceae), however, the principal active compound is the sesquiterpene lactone parthenin (Sukhada and Jayachandra 1980a, b), which has been dis-

cussed above in the context of host-plant location by specialist herbivores and pollen defense, and which evidence suggests might in part be contained in the pollenkitt.

Summary and future research directions

The inclusion of volatiles in pollenkitt appears to have evolved under different selection pressures related mainly to pollinator attraction and pollen protection. However, while a variety of functions can be proposed for pollen odor, we need to obtain a big picture of the relative importance that these two forces have had in the evolution of pollen odor emission, such as through comparative surveys of plants with different modes of pollination. Studies of pollen odor in plants pollinated by wind and by nonpollen-feeding animals, or showing special cases of pollination mentioned in the text, would complement the available studies on plants pollinated by pollen-feeding insects; these would also clarify the occurrence of particular volatiles, including α-methyl ketones, in relation to pollination.

Evidence from the handful of studies conducted to date proposes several functions that individual volatiles (singly or in mixtures) may serve in the interactions between pollen on the one hand, and pollinators, pollen predators, and pathogens, on the other hand, and these are summarized in Table 6. These can be divided into activities that modulate the behavior of pollen-feeding animals or that interfere with the growth and survival of pathogens and other pollen. This list is tentative and will undoubtedly be added to over time. The field is wide open for investigation; one notable unrepresented area that deserves attention is the possibility that pollen volatiles might be involved in some aspects of pollen-stigma interactions. Experimental attempts to clarify the functions of specific pollen volatiles have demonstrated that each volatile may play multiple roles and that effects of the pollen odor chemicals on other organisms are complex and specific to the particular pollen-organism interaction in question. This empha-

sizes the need to evaluate each species-species interaction on a case-by-case basis.

In the pollen-organism interactions involving animals, species-specific pollen odors can enhance the animal's ability to discriminate between pollen – and flowers – of different species and consequently increase its pollen-foraging efficiency. The volatile cues would be learned during foraging and would be perceived as the insect is selecting among available pollen sources while still in flight. For pollen-specialist insects, species-, genus- or even family-specific chemicals in the pollen odors can serve as key recognition chemicals and thereby allow the insects to specialize on a particular species or taxonomic group of plants. Host-plant recognition by the insect, especially prior to it having any foraging experience, would occur prior to or after alighting on the flower, depending on the volatility of the key chemicals and the distance at which they can be perceived by the insect to trigger a behavioral or physiological response. It would be interesting to investigate if pollen odors in species visited by specialist bees have chemical characteristics, such as the presence of taxon-specific defense volatiles, that set them apart from others. More chemical studies of pollenkitt constituents and improved approaches to pollen odor analyses are needed to identify compounds of low volatility (e.g. sesquiterpene lactones in Asteraceae) that could be of value in the olfactory (i.e. pre-feeding) discrimination of pollen by both generalist and specialist pollen feeders. When pollen odor forms a distinct contrast with odors from other floral parts, it offers pollen-foraging insects a pollen-cue that they can use, through variations in its intensity, to assess the amount of pollen reward available in individual flowers during their in-flight selection of flowers to visit. If the insect is a pollinator, these three effects can result in greater foraging efficiency and flower constancy, which can translate into increased pollination and reproductive success for the plant. However, if the insect is a pollen feeder that does not participate in pollination, any features of the pollen

Table 6. Summary of ecological functions attributed to pollen odors in the interactions between plants (flowers) and other organisms. See text for specific examples

Function	Timing of function	Advantage to plant	Advantage to other
Modulation of animal behavior			
Host-plant selection by generalist pollen feeders	pre-alighting	yes/no[a]	yes
Host-plant recognition by specialist pollen feeders	pre- and post-alighting[b]	yes/no[a]	yes
Assessment of pollen reward in the flower	pre-alighting	yes/no[a]	yes
Defense of pollen against pollen feeders	pre- and post-alighting[b]	yes	no
Interference with growth and survival in non-animals			
Defense of pollen against pathogens (fungi, bacteria)	at all times	yes	no
Pollen allelopathy[c]	on stigma	yes	no

[a] Depends on whether the pollen feeder is also a pollinator
[b] Depends on the volatility of pollen odor chemicals and the distance at which they are perceived
[c] Participation of pollen volatiles needs to be experimentally confirmed

odor that make it a more apparent target become a disadvantage to the plant. Comparison of feeding-deterrent chemistry in pollen and other floral parts (including anthers) would give insight into how defenses are spatially allocated within flowers and what impact this might have on pollination.

Interactions between pollen odor and non-animals seem to involve primarily interference, where pollen volatiles inhibit the growth and survival of pathogenic organisms. Pollen volatiles with activity would be present at all times in the odor, and the actual occurrence of such interference would depend on the encounter between pollen and pathogen and any conditions that would influence the pathogen's susceptibility to the volatiles. The inhibitory effects of pollen odor on pathogen growth is based mainly on circumstantial evidence. The antimicrobial activity of diverse floral – and pollen – volatiles has been demonstrated mainly in vitro, and conclusive evidence requires that pollen chemicals be tested in vivo against organisms that are ecologically significant to the plant species in question. It is also possible that defense volatiles might benefit bee pollinators that mass provision nest cells with pollen; chemicals that decrease pollen spoilage would result in higher larval survivorship and might be preferred as food sources by the next generation. With respect to the few document-

ed cases of pollen allelopathy, participation of pollen odor remains to be confirmed.

The field of pollen odor and elucidation of its adaptive significance is only at its beginnings and much more work lies ahead both for general investigations of pollen odor in relation to pollination and for specific studies of pollen odor chemicals in pollen-organism interactions. These will shed light on the selection pressures leading to the evolution of pollen odor emission and of the functions served by particular pollen volatiles.

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