

Pollen nutritional content and digestibility for animals

T. H. Roulston¹ and J. H. Cane²

¹Department of Entomology, North Carolina State University, Raleigh North Carolina, USA

²USDA-ARS Bee Biology and Systematics Lab, Utah State University, Logan, UT, USA

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Abstract. This paper reviews the literature concerning digestion and nutrient content of pollen. Four topics are addressed in detail: 1) The mechanism of pollen digestion by animals; 2) The efficiency of mechanical and digestive removal of pollen content by various animals; 3) Range and taxonomic distribution of pollen nutrients, and 4) Adaptive hypotheses proposed to associate pollen chemistry with pollinator reward. Studies on the mechanism(s) of pollen digestion remain inconclusive, but suggest that differences in digestibility among pollen types may reflect differences in pollen wall porosity, thickness, and composition. Although hummingbirds reportedly digest pollen very poorly, most animals studied, including those that do not regularly consume pollen, can digest 50–100% of ingested grains. Overlooked and recent research of pollen protein content shows that pollen grains may contain over 60% protein, double the amount cited in some studies of pollen-feeding animals. Adaptive hypotheses that associate pollen starch and pollen caloric content with pollinator reward remain unsubstantiated when critically viewed through the lens of phylogeny.

Key words: Pollen chemistry, pollen digestion, pollination syndrome, palynology, bees, nutrition.

Pollen grain digestion

Pollen walls so resist decay and digestion that they often survive intact and recognizable for

millions of years in bog and sediment deposits. Their durability lends paleoecologists a valuable tool for reconstructing ancient floras and paleoclimates. Their durability also presents pollen-feeding animals with a substantial digestive challenge: How to access the nutrient-rich cytoplasm encased within the refractory pollen wall?

Generalized structure of pollen grains

The outermost layer of the pollen wall is the pollenkit, a semi-solid coating comprised primarily of neutral lipids, hydrocarbons, terpenoids, and carotenoid pigments (Dobson 1988) (see also Nepi and Franchi, and Hesse, this issue). Inside the pollenkit is the exine (thin or lacking in some monocots, Kress et al. 1978), an often intricately-ridged matrix of the complex carbohydrate sporopollenin. The exine greatly resists decay and digestion, but is commonly perforated by one-to-several pores or slits (germination pores) that lead to the inner wall layer, known as the intine. The intine, composed primarily of cellulose and pectin, also resists decay and digestion, and forms the final barrier to the nutrient-rich cytoplasm. Thus, any animal consuming pollen contacts pollenkit nutrients through external probing of pollen grains or ingestion of pollen grains, but must penetrate or dismantle two resistant pollen wall layers in

order to access cytoplasmic nutrients. Six basic methods have been suggested for animals to extract pollen contents:

- I Crack open the pollen wall mechanically
- II Pierce the pollen wall with sharp mouthparts
- III Dissolve the pollen wall with enzymes
- IV Induce germination or pseudo-germination
- V Burst the pollen wall through osmotic shock
- VI Penetrate the pollen wall with digestive enzymes

The first three of these methods are rare, and the last three are difficult to distinguish. Some pollen-feeding beetles and micropterygid moths possess grinding mandibles said to function in grinding pollen (e.g. Meeuse 1961, Grinfel'd 1975, Crowson 1981, Barth 1985, Thien et al. 1985). We have been unable to find any study that demonstrates whole pollen entering and crushed pollen leaving the mandibles of any animal, however. Claims in the literature appear to originate from morphological analyses that correlate certain mandible structure with a pollen diet (e.g. Grinfel'd 1975, Crowson 1981, Mann and Crowson 1981, Thien et al. 1985). Mann and Crowson (1981) described the asperated or tuberculated mandibular mola of some primitive chrysomelid beetles as an adaptation to crushing pollen, a mechanism considered plausible, yet unsubstantiated, by Samuelson (1994). Studies of higher Chrysomelidae revealed unbroken pollen grains in the gut (Samuelson 1994). Mann and Crowson (1981) considered mandibular cracking of individual pollen grains to be common in beetles of the Nitidulidae, Cryptophagidae, Phalacridae, Anaspidae, Orsodacninae, Aulacoscelinae, Anthribidae, and Nemomychidae. Early claims that honey bees cracked pollen grains with their mandibles (e.g. Snodgrass 1925) were not borne out by subsequent analyses of pollen digestion (see below). Whitcomb and Wilson (1929) determined that honey bees could not generate sufficient crushing force with their mandibles to crack pollen grains.

Two studies of pollen-feeding beetles show that some beetles may crack pollen grains in

their digestive tract, rather than in their mouthparts. The beetle *Cyclocephala amazona* ingests both pollen and lignified trichomes from the inflorescence of the peach palm, *Bactris gasipaes* (Rickson et al. 1990). The trichomes, of no nutritive value, apparently act as gastroliths that crush pollen grains in the beetle's digestive tract. The boll weevil also breaks pollen grains, but by an undescribed method. Cate and Skinner (1978) found that pollen grains that passed through boll weevils in less than two hours were defecated whole, whereas pollen grains retained for longer periods of time were more likely to be fragmented.

Numerous species of thrips (Thysanoptera) feed on pollen (reviewed in Kirk 1984). The diameter of the pollen grains of most species (15–60 μm , Simpson and Neff 1983) exceeds the breadth of the maxillary feeding channel of most thrips. Rather than ingesting whole pollen grains, thrips position themselves in front of individual grains, pierce the exine with their mouthparts, and suck out the contents, leaving behind collapsed or air-filled grains (Kirk 1984, Grinfel'd 1959, cited in Kirk 1984). This process takes from 2–20 seconds per grain, depending on size of grain, temperature, and thrips species. Thrips apparently do not need to pierce grains at particular positions, such as a pore, but may have to pierce large grains at multiple positions in order to extract all of the contents (Kirk 1984).

The only other organisms reported to pierce pollen grains and extract their contents without ingesting the grains are some species of ceratopogonid flies (Downes 1955, De Meillon and Wirth 1989). Female Ceratopogonidae ("biting midges," "no-see-ums") typically feed on the blood or hemolymph of living mammals, birds, or insects prior to laying eggs. Females of *Atrichopogon pollinivorus*, in contrast, perch on an anther or stigma, press the tip of their rostrum against a pollen grain, and extract the pollen contents in ca. 6 seconds (Downes 1955). Downes reported *Atrichopogon* spp. consuming the pollen of *Lonicera* sp., *Crataegus* sp., and *Prunus lusitanica*. He fur-

ther observed that the ovaries of pollen-foraging *Atrichopogon* spp. were small and undeveloped, similar in appearance to blood and hemolymph-consuming species of ceratopogonids prior to a blood meal, leading him to conclude that pollen consumption served to mature ceratopogonid ovaries and oocytes. Although male ceratopogonids were often observed sipping nectar on the same flowers as females, they were never observed consuming pollen.

Some Collembola ("springtails") are the only animals known to fully digest the pollen wall. Scott and Stojanovich (1963) fed *Juniperus* pollen to the semi-transparent collembolan *Onychiurus pseudofimetarius* and observed that the pollen wall ruptured then completely disappeared during its passage through the digestive tract. Not all collembolans, however, possess this digestive ability. Pollen walls remained intact while passing through the gut of *Entomobrya socia* (Waldorf 1981).

The discrete methods of pollen nutrient extraction described above account for half of the methods hypothesized, but they apply to very few of the organisms known to digest pollen. Most animals are thought to extract pollen nutrients through pollen germination (or pseudo-germination), osmotic shock, and/or penetration of the pollen wall by digestive enzymes. Pollen grains of many species will germinate in a 10% sucrose medium in the presence of micronutrients such as boric acid. As pollen germinates, it releases proteins (Stanley and Linskens 1965) and free amino acids (Linskens and Schrauwen 1969). Similarly, pollen pre-soaked in a 10% sucrose medium then placed in acid may form instant pollen tubes (IPoTs, a process hereafter referred to as pseudo-germination), similar in appearance to naturally germinating pollen but arising more suddenly (Linskens and Mulleneers 1967). Most pollen-feeding animals sip nectar while ingesting pollen, and could retain pollen in a nectar-filled, weakly acidic portion of the digestive tract (Turner 1984). Thus, they may subject pollen to conditions in which it either germinates or pseudo-germi-

nates, thereby extracting both free amino acids and proteins without breaking or penetrating the pollen grain.

Pollen-feeding *Heliconius* butterflies add nectar to a collected pollen mass, churn the mass externally for several hours with their proboscis, and ingest pollen-derived nutrients without ingesting whole pollen grains (Gilbert 1972). These nutrients greatly increase egg production and longevity in *Heliconius*. The eucalypt nectar fly, *Drosophila flavohirta*, also gathers a large pollen load on the tip of its proboscis and agitates it, without ingesting the pollen grains (Nicolson 1994). Nicolson considered this to be a nutrient gathering strategy similar to *Heliconius*. In both cases, the nutrients reportedly become available through nutrient release of pollen grains undergoing germination, rather than through pollen grain degradation.

The syrphid fly *Cheilosia albitarsis* ingests both the pollen and nectar of its host plant, *Ranunculus repens* (Haslett 1983). The pollen remains intact as it passes through the oesophagus and crop. As pollen reaches the midgut, however, most grains extrude their cytoplasm through a germination pore in a manner reminiscent of germination or pseudo-germination (Haslett 1983). Most pollen grains are empty by the time they reach the hindgut. Pollen grains in the crop of the oedemerid beetle *Stenostoma coerulea* show similar cytoplasmic protrusions and are empty when they reach the hindgut (Crowson 1981). The honey possum, *Tarsipes rostratus*, digests 90–100% of the *Banksia* pollen it ingests (Turner 1984). Turner found that some pollen in the lower stomach showed cytoplasmic protrusions similar to germinating pollen tubes, but Richardson et al. (1986) did not observe such protrusions.

Various flower-visiting bird species ingest pollen incidentally or intentionally while imbibing nectar at flowers. A species of Darwin's finch, *Geospiza scandens*, feeds cactus (*Opuntia echios*) pollen to its offspring prior to the onset of seasonal rains, a time of good pollen availability but poor alternative forage (Grant

1996). Grant found that *G. scandens* (and the related *G. fortis*) digested approximately 90% of pollen grains consumed. She demonstrated that most *O. echios* pollen will germinate in a solution of *O. echios* nectar and water, and hypothesized that the simultaneous ingestion of *O. echios* pollen and nectar led to pollen germination and subsequent digestion. Grant (1996) found that 11.5% of pollen in the crop of nestlings showed signs of germination. In contrast, Paton (1981) found that *Banksia* pollen grains germinated in a sugar solution in vitro, but not when consumed with sugar solution by the New Holland Honeyeater, *Phylidonyris novaehollandiae*.

Pollen digestion has been studied most extensively in bees, but a complex and somewhat confusing set of interpretations has emerged. Adult bees possess a crop, in which nectar and pollen may mix, thus providing a pre-treatment that could lead to germination or pseudo-germination. The crop leads through the proventricular valve to the gut, a region that differs substantially in osmotic pressure from the crop. Thus, pollen consumed by adult bees is subjected to immersion in a sugar solution followed by an abrupt osmotic gradient. In contrast, larval bees have no crop. Pollen enters the gut without internal exposure to nectar sugars or sudden changes in osmotic pressure. The pollen consumed by larval bees, however, is part of a food provision including a large quantity of nectar. Thus, larval pollen has been exposed to a liquid sugar environment prior to ingestion. For most bee species, larvae consume the pollen within a few days of pollen collection. For honey bees, however, pollen may be stored in the hive for an extended period of time. Adult honey bees add secretions that inhibit pollen germination (Klungness and Peng 1983). Additionally, the stored pollen undergoes considerable biochemical change due to the activities of microbial organisms. Thus, there may be differences in pollen digestion between larvae and adult bees, and among bees that differ substantially in sociality.

Kroon et al. (1974) noted that pollen grains will burst when transferred suddenly

between chambers that differed in osmotic pressure. These researchers determined that the osmotic differential between the honey bee crop and ventriculus was sufficient to burst pollen grains, and suggested that the burst grains then traveled through the gut where their contents were accessible to digestive enzymes. They hypothesized that this "osmotic shock" was a necessary prerequisite to digestion. Peng et al., however, (1985, 1986) did not find burst pollen grains in the anterior midgut of the honey bee. Instead, these researchers found that *Medicago sativa* (alfalfa) and *Taraxacum officinale* (dandelion) pollen slowly degraded and lost their contents during their passage through the midgut. *Taraxacum* pollen swelled at the germination pores in the bee's anterior midgut, but showed no further signs of germination (Peng et al. 1985). The pollen walls of *Taraxacum* gradually became misshapen as the cytoplasm drained through the germ pores. *Medicago* pollen showed germination-like cytoplasmic protrusions, still contained by the bulging intine, in the middle midgut (Peng et al. 1986). As the *Medicago* pollen reached the posterior midgut, the pollen wall partially broke down and the protruding intine ruptured, releasing the cytoplasm. Peng et al. (1986) hypothesized that the digestion process comprised enzymatic degradation of the pollen wall, followed by extrusion of the cytoplasm due to osmotic pressure, weakening of the intine by proteases, and eventual rupture of the intine by continued osmotic pressure. In this interpretation, cytoplasmic protrusions resembling germination are caused by cell wall breakdown and osmotic pressure rather than induction of germination. Although pollen wall components such as sporopollenin and cellulose are unlikely to be digested by honey bee digestive enzymes, Klungness and Peng (1984) found that honey bees could partially digest the hemicellulose and pectic acid components.

Cruz-Landim and Serrao (1994) and Serrao and Cruz-Landim (1996) studied pollen digestion in adult and larval stingless bees of the genera *Trigona* and *Scaptotrigona*. With

both larvae and adults, they found that pollen contents were digested without bursting of the exine. They inferred pollen digestion to occur as the result of bee digestive enzymes altering the pollen wall, followed by penetration into the pollen grain by digestive enzymes, then the bursting of the intine and release of pollen contents through osmotic pressure.

Suárez-Cervera et al. (1994) studied pollen digestion by larvae of solitary bees in the genus *Osmia*. They compared pollen from unconsumed portions of the provision mass with pollen consumed and defecated by mature larvae. The pollens with thin intines showed cytoplasmic protrusions at the germination pores in the provision mass (*Cistus* sp. and *Sonchus* sp.); these were better digested than pollens with relatively thick intines that showed no modification while in the provision (*Prunus* sp. and *Quercus* sp.).

Dobson and Peng (1997) studied pollen digestion in 2–3 week old larvae of the solitary, pollen-specialist bee, *Chelostoma florissomne*. By staining serial gut sections for protein, carbohydrates and lipids, Dobson and Peng determined the extent and location of digestion, as well as the condition of the pollen wall as it passed through the digestive tract. They found no evidence of pollen germination. They found that pollen grains gradually emptied their contents through the germination pore, without breaking their walls before losing most of their contents.

It is difficult to assemble all of these observations into a general mechanism of pollen digestion because different observations have been made on different organisms and different life stages with different pollens. No studies have been carried out that intentionally varied pollen characteristics in order to assess their effect on digestion. Dobson and Peng (1997) provide a lucid synthesis of pollen digestion studies on bees, which we will briefly summarize here. The intine that covers germination pores provides the greatest barrier to penetration of the pollen grains by insect digestive enzymes. For larvae of bee species other than honey bees, this layer may be

disrupted by germination-inducing conditions in the sugary pollen provisions. For bee adults, this layer may be disrupted at the germination pores by osmotic shock as the pollen passes from the crop through the proventricular valve. As the pollen passes through the gut, digestive enzymes degrade the hemicellulose and pectic acid components of the intine, further allowing pollen grain contents to flow out as insect digestive enzymes flow in. The digestion of particular compounds, such as starch, appears to depend on their removal from the pollen grain. Reported discrepancies regarding the extent to which pollen walls are broken probably arise from different chemical characteristics of the walls of different pollens.

Researchers studying pollen digestion by animals generally attribute changes in the pollen wall, aside from a slight swelling at the germination pores, to the action of animal-derived digestive enzymes. Pollen cell walls may be altered by pollen enzymes already present in the cell wall, however. During the initial stages of germination, the sporopollenin of the spines of *Pharbitis nil* pollen degrades into an amorphous layer (Gherardini and Healey 1969). Gherardini and Healey (1969) report on studies that show that hydrolytic enzymes are present in the intine and are particularly active near the germination pores. When pollen is placed on a stigma, even a non-congeneric stigma, cell wall degradation may begin without the appearance of a germination tube or other signs of germination. Thus, an absence of overt signs of pollen germination in the crop or gut of a pollen-feeding animal does not necessarily mean that important endogenous changes in pollen wall chemistry associated with germination-induction conditions have not already occurred.

Grain emptying efficiency of various animals

Digestive efficiency has been calculated as percent empty pollen grains in the feces of numerous animals (Table 1). Some researchers adjusted the efficiency to accommodate the percentage of empty grains pre-ingestion,

Table 1. Comparison of percent of pollen grains emptied by passing through the digestive tract of various animals

Animal group	Animal species	%empty	Plant species	Reference
Vertebrates				
Mammals				
Rodents	<i>Aethomys namaquensis</i>	60.4		Vantets 1997
	<i>Elephantulus edwardsii</i>	50.3		Vantets 1997
	<i>Mus minitoides</i>	83.0		Vantets 1997
	<i>Rhabdomys pumilo</i>	60.4		Vantets 1997
Bats	<i>Syconycteris australis</i>	53–57	<i>Banksia</i>	Law 1992b
	<i>Sturnira lilium</i>	47	columnar cactus	Herrera and Martínez del Rio 1998
		27	<i>Pseudobombax ellipticum</i>	
	<i>Artibeus jamaicensis</i>	71	columnar cactus	Herrera and Martínez del Rio 1998
		53	<i>Pseudobombax ellipticum</i>	
		32	<i>Hylocereus undatus</i>	
	<i>Anoura geoffroyi</i>	75	columnar cactus	Herrera and Martínez del Rio 1998
		82	<i>Pseudobombax ellipticum</i>	
		50	<i>Hylocereus undatus</i>	
	<i>Leptonycteris curasoae</i>	86	columnar cactus	Herrera and Martínez del Rio 1998
Marsupials	<i>Acrobates pygmaeus</i>	78	<i>Eucalyptus</i> sp.	Turner 1984
	<i>Petaurus australis</i>	60–70	<i>Banksia/Eucalyptus</i>	Quin et al. 1996
	<i>Tarsipes rostratus</i>	78.6	<i>Banksia integrifolia</i>	Turner 1984
		65.0	<i>Banksia serrata</i>	Turner 1984
		63.7	<i>Banksia spinulosa</i>	Turner 1984
Birds	<i>Phylidonyris novaehollandiae</i>	0		Paton 1981
		41 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Melopsittacus undulatus</i>	42 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Poephila guttata</i>	41 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Geospiza scandens</i>	>90 ^a	<i>Opuntia echios</i>	Grant 1996
	<i>Geospiza fortis</i>	>90 ^a	<i>Opuntia echios</i>	Grant 1996
	<i>Calypte anna</i>	3.8	<i>Zauschneria</i> sp.	Brice et al. 1989
		4.7	<i>Callistemon</i> sp.	Brice et al. 1989
		6.9	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Calypte anna</i> (nestling)	5.4	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Calypte costae</i>	0	<i>Zauschneria</i> sp.	Brice et al. 1989
		0	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Trichoglossus haematodus haematodus</i>	4.5	<i>Eucalyptus</i> sp.	Brice et al. 1989

Table 1 (continued)

Animal group	Animal species	%empty	Plant species	Reference
Invertebrates Bees	<i>Trichoglossus h. moluccanus</i>	6.6	<i>Eucalyptus</i> sp.	Brice et al. 1989
		12.9	<i>Prunus</i> sp.	Brice et al. 1989
	<i>Trichoglossus h. m.</i> (nestling)	26	<i>Eucalyptus</i> sp.	Brice et al. 1989
		12.3	<i>Prunus</i> sp.	Brice et al. 1989
	<i>Nymphicus hollandicus</i>	18.1	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Nymphicus hollandicus</i> (nestling)	38.0	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Apis mellifera</i> (adults)	50		Cruz-Landim 1985
	<i>A. mellifera</i> (1 day old adults)	98.2 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (1 day old adults)	72.6	<i>Trifolium</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (9 day old adults)	96.0 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (9 day old adults)	93.1	<i>Trifolium</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (23 day old adults)	49.5 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (23 day old adults)	62.0	<i>Trifolium</i>	Crailsheim et al. 1992

^a(%empty grains in feces – %empty grains in pollen prior to consumption)/%full grains prior to consumption

which can be substantial (e.g. Paton 1981), while other researchers did not account for this factor. In Table 1 we report digestive efficiency adjusted for pre-ingested grains whenever sufficient data were available. Efficiency ranged from nearly zero for some bird species (hummingbirds, *Calypte* spp., and the honey-eater *Phylidonyris novaehollandiae*) to 80–100% for some bat species, a pollen-feeding Darwin's finch, and the mouse *Mus minutoides* fed a laboratory diet of pollen. Honey bees extracted pollen contents from 50–98% of ingested pollen, depending on pollen type and age of the bee. Table 1 shows that various facultative pollen-feeders can extract the cytoplasm of more than half of the pollen grains they consume. Digestive efficiencies among pollen-feeders consuming different pollens cannot readily be compared because of differences in the pollen consumed. The bat *Artibeus jamaicensis* extracted the cytoplasm of 72% of the pollen grains of an unknown columnar cactus, but only 32% of the cytoplasm of the pollen grains from the cactus *Hylocereus undatus* (Herrera and Martínez del Río 1998). It is interesting to note that some animals for

which pollen might not be a regular part of the diet, such as mice and some bats, can efficiently extract pollen cytoplasm. Herrera and Martínez del Río (1998) found, however, that bat species that regularly consume pollen can digest it more efficiently than bat species that do not regularly consume pollen.

Nutritional content of pollen

Various authors reviewing pollen chemistry have reported the known range or average concentration of nutrients in pollen grains (e.g. Lundén 1956, Barbier 1970, Stanley and Linskens 1974, Solberg and Remedios 1980, Nepi and Franchi, this volume). Nutrients reported include protein, nitrogen, amino acids, starch, sterols, and lipids. Most analyses and summaries are based on few taxa [the most extensive survey cited usually being that of Todd and Bretherick's (1942) analysis of 31 plant species]. Chemical analyses of pollen are difficult because most analytical techniques require more pollen than can be easily collected by hand. Thus, two categories of plants have generally been targeted for analysis: 1) ane-

mophilous (wind-pollinated) trees, such as *Pinus* and *Quercus*, that produce abundant pollen, and 2) entomophilous (insect-pollinated) or anemophilous species whose pollen is readily collected by honey bees and subsequently removed from honey bees by researchers (e.g. Todd and Bretherick 1942, Vivino and Palmer 1944, Standifer 1966, Ibrahim 1974, Standifer et al. 1980, Rabie et al. 1983, Schmidt and Johnson 1984). This has led to a good understanding of the pollen chemistry of a small number of anemophilous trees and a very imperfect understanding of the composition of honey bee-collected pollens. Honey bees mix pollen with regurgitated nectar or honey for transport on their legs. Thus, the "pollen pellets" often subjected to chemical analyses contain both pollen and nectar or honey. The fraction of pollen pellets attributable to added nectar or honey has never been estimated directly. The data set of Todd and Bretherick (1942) comprised both hand and honey bee-collected samples, including one species, *Pinus contorta*, that was collected by both techniques. Their work indicates that most reducing sugars present with pollen transported by honey bees are derived from added nectar or honey; these reducing sugars may account for up to 40% of the dry weight of pollen pellets ($\bar{x} = 25.7 \pm 5.19\%$, $n = 26$, honey bee-collected, $\bar{x} = 2.59 \pm 3.59\%$, $n = 6$, hand-collected). Because the chemical constituents of pollen grains are generally reported on a per weight basis, any analysis of pollen pellets that does not account for the added weight of nectar or honey sugars will greatly underestimate the concentration of the chemical constituents in the pollen itself. Comparisons of pollen protein estimates from nine species analyzed from both hand and honey bee-collected pollens led Roulston et al. (in Press) to conclude that the bias created by added sugar was extremely variable and could not be removed by a standardized multiplier. At present, we know nothing about the factors governing the amount of sugar honey bees add to transported pollen (i.e. if it depends on the properties of the pollen or merely depends on

the sugar concentration of the nectar or honey in the honey bee's crop). Most chemical analyses of pollen are based on honey bee-collected pollen. Thus, there is great uncertainty for the majority of reported values. While we may know the concentration of pollen constituents to the correct order of magnitude (assuming that none of the nutrients originate from nectar or the honey bee itself, as in reducing sugars), we often do not have sufficient data to compare chemical constituents across taxa or pollination modes for pollen that is taken from foraging honey bees.

Pollen nitrogen/protein content

Numerous studies have estimated the nitrogen/protein content of pollen (e.g. Lidforss 1899, Todd and Bretherick 1942, Nielsen et al. 1955, Levin and Haydak 1957, Knight et al. 1972, Howell 1974, Loper and Berdel 1980, McCaughey et al. 1980, Solberg and Remedios 1980, Standifer et al. 1980, Rabie et al. 1983, Buchmann 1986, Schmidt et al. 1987, Roulston et al., in Press). Data from hand-collected samples indicate that pollen ranges from 0.36% N for *Cupressus arizonica* to 9.7% N for *Dodecatheon clevelandii* (Buchmann 1986). The two most commonly-used techniques for estimating nitrogen, micro-Kjeldahl acid digestion and combustion, generally produce similar results (Buchmann 1986, Roulston et al., in Press).

Most estimates of crude protein concentration in pollen are derived from estimates of nitrogen concentration multiplied by 6.25. This conversion factor is well established for estimating animal protein mass from tissue such as muscle, but may not be fully accurate for plant tissues. Milton and Ditzis (1981) calculated a nitrogen-protein conversion factor of 4.4 for tropical leaves eaten by howler monkeys. Solberg and Remedios (1980) calculated conversion factors of 4.0 and 3.8 respectively for hand-collected and bee-collected pollens, but Rabie et al. (1983) suggested that those conversion factors failed to adequately

account for ammonia nitrogen. Rabie et al. (1983) analyzed honey bee-collected pollen from 21 plant species and suggested using a standard conversion factor of 5.6. Preliminary work with hand-collected pollens indicated that the conversion factor may be as low as 4.5–5.25 (Buchmann 1986). Thus, the common use of a 6.25 conversion factor may overestimate actual crude pollen protein concentration, but should provide accurate relative estimates when comparing plant species that have been analyzed by comparable techniques. Roulston et al. (in Press) showed that a modified Bradford Assay using *Typha latifolia* pollen as a protein standard produced similar pollen protein concentration estimates to those from micro-Kjeldahl and combustion.

Using the 6.25 conversion factor for the subset of raw N values, Roulston et al. (in Press) compiled a data base of pollen protein concentrations from 377 plant species in 93 plant families. They found that pollen protein concentration ranged from 2.5–61% protein and was highly conserved within plant genera and families. The plants poorest in pollen protein were anemophilous gymnosperms, such as Cupressaceae and Pinaceae, while those richest in protein were vibratile-pollinated herbs such as *Dodecatheon* (Primulaceae), *Rhexia* (Melastomataceae), and *Solanum* (Solanaceae), as well as bat-pollinated Bombacaceae and bird-pollinated Campanulaceae. Although many anemophilous pollens were protein poor, evolutionary shifts to anemophily were not statistically associated with decreased protein concentration (Roulston et al., in Press). Roulston et al. found no evidence that bee species preferentially collected pollen that was particularly rich in protein.

Animals collect pollens that may differ widely in protein content. Protein concentration may influence the frequency or absolute number of foraging trips an animal must make in order to provide offspring with sufficient pollen to meet their dietary protein requirements (Danforth 1990, Neff and Simpson 1997, Roulston and Cane submitted). Although some authors have stated that crude

protein estimates do not measure pollen quality (e.g. Todd and Bretherick 1942), no other chemical constituent of pollen grains has been shown to influence as many aspects of a pollen consumer's performance as well as protein. Levin and Haydak (1957) transplanted eggs of the solitary bee *Osmia lignaria* onto honey bee-collected pollens held in separate vials. Of the ten pollens tested, only the five richest in protein supported development to the adult stage. Diet supplementation with vitamin-free casein, yeast, and egg yolk indicated that protein, B-vitamins, and sterol content all influenced bee development. Schmidt et al. (1987) fed 25 pollens to adult honey bees and found that longevity increased with protein concentration of the pollen and total protein consumed. As noted by Schmidt et al. (1987) and Roulston and Cane (submitted), bees do not collect/consume equal amounts of all pollens. Thus, total protein consumed reflects both the protein concentration of the pollen and the amount of pollen consumed. Bees apparently do not prefer to collect protein-rich pollens (Van Der Moezel et al. 1987, but see Schmidt and Johnson 1984).

Bee body size varies with protein consumed. Regali and Rasmont (1995) found that larval nestmates fed one pollen diet by worker bumble bees grew larger than those fed a second pollen diet that was poorer in protein. Greenberg (1982) and Roulston and Cane (submitted) increased the size of *Lasioglossum zephyrum* offspring by supplementing an adequate but protein-poor diet of *Typha latifolium* pollen with soy protein powder. Levin and Haydak (1957, data plotted from Levin and Haydak's Table 4) and Roulston and Cane (submitted) showed that adult bee body size increased linearly with total protein consumed.

Atypical of bee species, honey bee workers monitor the growth of developing larvae and feed them periodically with a diet rich in glandular secretions that originate in the hypopharyngeal glands. The hypopharyngeal glands develop to a great extent after adult emergence, reaching their maximum size after five or more days (Winston 1987). Standifer

et al. (1967) found that the development rate of worker hypopharyngeal glands increased with more dietary protein. Herbert (1992), however, cautioned that the size of the hypopharyngeal glands did not always correspond to the production of glandular secretions adequate for larval growth.

Protein digestion

Pollen protein digestion has been studied in few animals. Pollen protein was efficiently digested by both the larvae of the solitary bee *Chelostoma florissomne* (Dobson and Peng 1997) and adult honey bees (Klungness and Peng 1984), with digestion initiated in the anterior midgut. Rats digested 52–59% of the pollen protein in a diet of pellets of *Eucalyptus* pollens taken from honey bees (Bell et al. 1983), while mice fed *Prosopis velutina* pollen digested 80% of pollen protein (Schmidt et al. 1984). Franchi et al. (1997) studied pollen protein digestion in a test apparatus, including pancreatic enzymes, designed to mimic the human digestive system. They found that after 24 hours, only 48% (*Papaver rhoeas*) and 59% (*Corylus avellana*) of pollen protein had been digested. These researchers concluded that the value of pollen as a human dietary supplement was greatly overestimated by simple analyses of pollen chemistry.

Nitrogen assimilation efficiency

Nitrogen assimilation efficiency has been determined for two bee species, *Megachile rotundata* (*M. pacifica*) and *Apis mellifera*. Wightman and Rogers (1978) calculated the nitrogen in the diet, feces, cocoons and mature larvae of *Megachile rotundata* and concluded that the bees assimilated 87.2% of ingested nitrogen. Schmidt and Buchmann (1985) restricted a honey bee colony to its hive in a small flight cage for 4 weeks. They determined the mass of nitrogen consumed and defecated by the entire colony and estimated that the colony assimilated 77–83% of ingested nitrogen. Such

efficient nitrogen assimilation is unparalleled among invertebrates (Wightman and Rogers 1978), a surprising fact given the inherent difficulty of extracting the nitrogen from the pollen grain. Not all bee species, or not all bees on all diets, assimilate nitrogen this efficiently, however. The bee *Osmia lignaria* retained only 35–50% of dietary nitrogen in adult body tissue (Levin and Haydak 1957). This figure excludes nitrogen in cocoons, a very minor nitrogen sink for *Megachile rotundata* (Wightman and Rogers 1978).

Pollen-feeding mammals also efficiently utilize pollen nitrogen. The marsupial *Petaurus breviceps* digested 76% of the nitrogen in its *Eucalyptus* pollen diet and maintained a daily nitrogen balance with the lowest input nitrogen of any marsupial yet recorded (Smith and Green 1987). The Queensland Blossom Bat, *Synconycotis australis*, digested 58–97% of the nitrogen in its laboratory pollen diet (Law 1992a).

Amino acid composition

Most pollens contain all the common amino acids (Solberg and Remedios 1980), but pollen sometimes lacks phenylalanine, tryptophane, hydroxyproline, tyrosine and aminobutyric acid (Johri and Vasil 1961). Tryptophane and phenylalanine are the only essential amino acids sometimes lacking (Lundén 1956). Pollen amino acids that are abundant in the free state also tend to abound in protein (Stanley and Linskens 1974). Solberg and Remedios (1948) reported that the six most common amino acids, aspartic acid, glutamic acid, proline, leucine, lysine, and arginine, comprised 60% of the protein weight of pollen grains. Among free amino acids, proline is often the most abundant and can account for 1–2% of the total weight of pollen grains (Stanley and Linskens 1974).

Although numerous studies have analyzed the amino acid contents of one or a few pollens (e.g. Nielsen et al. 1955, Gilliam et al. 1980, McCaughey et al. 1980, Solberg and Remedios 1980, Standifer et al. 1980, Ceausescu and Mosoiu 1981, Rayner and Langridge 1985, Wu

and Murry 1985, Buchmann 1986, Loper and Cohen 1987, Agarwal and Nair 1989, Erhardt and Baker 1990, Clark and Lintas 1992), no study has yet assembled sufficient data to discern taxonomic and ecological trends. Thus, it is not clear if a lack of generalities concerning differences in amino acid content are due to a lack of broad trends or a lack of data. Based on small data sets, Solberg and Remedios (1980) and Lundén (1956) concluded that pollen of wind and bee-pollinated plants contained similar amino acid profiles. McLellan (1977) compared the concentrations of 16 amino acids among seven honey bee-collected pollens. He found significant differences among species for serine, cystine, and histidine, but did not speculate on the causes of those differences.

Honey bees require ten amino acids for maximum development: arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine (De Groot 1953). Honey bees commonly collect the pollen of dandelion, *Taraxacum officinale* (e.g. Peng et al. 1985), which lacks the essential amino acids tryptophane and phenylalanine (Auclair and Jamieson 1948) and is deficient in arginine (Herbert 1992). Young honey bees fed only dandelion pollen failed to rear brood (Herbert et al. 1970). Dandelion pollen amended with L-tryptophane, L-phenylalanine, and L-arginine promoted full brood rearing (Herbert 1992), but the addition of L-tryptophane and L-phenylalanine alone (Herbert 1992) or L-arginine alone (Loper and Berdel 1980) failed to do so. Adult honey bees also died sooner on a diet of dandelion pollen than on other pollens (Knox et al. 1971).

No extensive studies exist for the amino acid requirements of pollen-feeding animals other than the honey bee. Amino acid requirements are similar for animals as varied as dogs, rats, pigs, humans, and bees (Howell 1974). Thus, it seems likely that most pollen-feeding animals will have similar amino acid requirements. Howell (1974) characterized the amino acids of two species of bat-pollinated plants and found them to be similar to those of bee-

pollinated plants. She speculated that the apparently rich concentration of proline, an important component of collagen, and tyrosine, perhaps a growth stimulant for young bats, in *Agave* spp. and *Carnegiea gigantea* pollen represented biochemical adaptations of the plant to attract their bat pollinators. Such speculation seems premature, however. There is insufficient evidence to distinguish the amino acid profile of any ecological group of plants, such as anemophilous, entomophilous, or chiropterophilous (bat-pollinated) from any other group of plants. Although *Agave* spp. and *Carnegiea gigantea* pollen appear to be rich in tyrosine, they are poorer in proline than various anemophilous plant species (see Solberg and Remedios 1980).

Starch content

Todd and Bretherick (1942) and Roulston and Buchmann (in Press) quantified starch content of 89 plant pollens. Average starch concentration ranged from 0–22% by dry weight. The starchiest pollens were *Typha latifolia* (22.3%), *Zea mays* (20.1%), *Salix* sp. (12.3%) and *Plantago lanceolata* (11.1%) (Roulston and Buchmann in Press), but 41 of the 89 pollens contained less than 1% starch (Roulston and Buchmann, unpublished). Calvino (1952), Baker and Baker (1979, 1983), and Franchi et al. (1996) scored pollen grains categorically as starchy or starchless, based on iodine staining, for ca. 1000 plant species per study. Calvino (1952) and Baker and Baker (1979, 1983) noted that starchy pollen occurred disproportionately often among anemophilous species. Both studies also noted that entomophilous pollens appeared more oily than anemophilous pollens, although neither study quantified lipid content.

Baker and Baker (1979, 1983), and later Grayum (1985), argued that starchy pollen arose as an adaptation to deter pollen theft by non-pollinating insects and/or lipid-rich pollen arose as a plant adaptation to attract and reward pollen-feeding pollinators. Their arguments assume that 1) pollens of plant species pollinated by non pollen-feeding vectors (e.g.

wind, birds, moths) are more likely to be starchy than plant species pollinated by pollen-feeding animals, and 2) pollen-feeding animals prefer non-starchy pollen.

Statistical support for the prevalence of starchy pollen in anemophilous lineages may depend on the statistical techniques applied. Treating every species as an independent sample, Baker and Baker (1979) concluded that the sample of anemophilous species (as well as groups pollinated by birds and lepidopterans) contained a greater proportion of starchy species than the sample of all ca. 1000 taxa analyzed. Roulston and Buchmann (in Press) tested this hypothesis with a sample of 207 plant species arranged into a phylogeny based on published cladograms. Using phylogeny-based statistics, they concluded that starchy pollen was not unusually associated with anemophilous lineages or any lineage in which pollen was not considered a pollinator reward. They concluded that the discrepancy between their results and those of Baker and Baker (1979) resulted from the prevalence of starchy species in three speciose, primarily anemophilous clades that were statistically treated as three independent samples by Roulston and Buchmann but numerous independent samples by Baker and Baker.

No experiments evaluate the preference of pollen-feeding animals for starchy or starchless pollen. Baker and Baker (1979) predicted that pollen-feeding insects would eschew starchy pollen because prior studies reported that honey bees have difficulty digesting starch. More recent work shows that honey bees and other bees can digest starch if they can free it from the amyloplasts and pollen grain (see below). Furthermore, the starchiest pollens known are either often collected by bees (e.g. *Zea mays*) or often used for the rearing of some bees species in the laboratory (e.g. *Typha latifolia*) (Roulston and Buchmann in Press).

Starch digestion

Digestion of pollen starch by bees varies among bee species and/or pollen types. Larvae

of *Chelostoma florissomne* (Dobson and Peng 1997) and *Osmia* spp. (Suárez-Cervera et al. 1994) efficiently digested the pollen starch of their respective host plant pollens, *Ranunculus* spp. and *Cistus* sp. *Osmia cornuta* completely digested the starch of *Euphorbia helioscopia*, but only partially digested the starch of *Ranunculus* sp. (Nepi et al. 1997). Honey bee adults readily digested starch when it was released from the pollen grain but only poorly or not at all in pollen grains that were not degraded during the digestive process (Klungness and Peng 1984). Thus, the presence of starch in the feces of honey bee adults (e.g. Parker 1926) may be due not to an inability to digest starch but rather to an inability to free it from the pollen grain. In *Chelostoma florissomne* larvae, pollen starch was released into the digestive tract (in the median midgut) considerably further along than where protein and lipid digestion began (Dobson and Peng 1997). Differences in starch digestion among pollen types could reflect different storage locations within the pollen grain or different chemical forms of starch. Franchi et al. (1997) found that starch-like short chain polysaccharides dispersed in the cytoplasm of *Papaver rhoeas* pollen were much more readily digested than the starch grains located in amyloplasts of *Corylus avellana* pollen when submitted to simulated human digestion.

Lipid content

Lipid content of pollen grains has most often been estimated through ether extraction, which removes 1–20% of the weight of pollen grains (Stanley and Linskens 1974). Ether extracts may include fats, fatty acids, some vitamins, pigments, higher alcohols, waxes, sterols and saturated hydrocarbons (Solberg and Remedios 1980). Solberg and Remedios (1980) concluded that the main ether extractable constituents of 16 hand-collected pollens were linolic, linoleic, and arachidonic acids that occurred in the pollenkitt. Dobson (1988) determined the number and class of pollenkitt lipids for 69 angiosperm species based on benzene then chloro-

form: methanol extraction followed by thin layer chromatography. Dobson (1988) noted that species pollinated by mere nectarivores contained fewer kinds of pollen-kitt lipids than species pollinated by bees, presumably because scents in the pollen-kitt attract bees seeking pollen. If the role of pollen-kitt were primarily one of pollinator attraction, then it might be expected that pollens of plants not pollinated by pollen-feeding organisms would contain less pollen-kitt than pollens of plants pollinated by pollen-feeders such as bees, bats, and some flies. An alternate function of pollen-kitt is to stick pollen on the body of animal pollinators so that it can be transported to conspecific flowers. Hesse (1979a, b, c) observed that anemophilous pollens tend to have less pollen-kitt and less sticky surfaces than closely-related zoophilous species. Thus, it might be expected that zoophilous pollens in general would have more pollen-kitt than anemophilous pollens, regardless of whether or not the pollen is consumed by pollinators. If pollen-kitt abundance corresponds to sticking pollen grains to animal pollen vectors and, additionally, attracting pollen vectors that also feed on pollen, we might predict that lipid abundance of pollen would follow this approximate pattern: anemophilous < zoophilous < pollen-feeding zoophilous.

This relationship is not apparent in a summary of lipid extractable materials for various plant taxa. Table 2 compiles published data for 62 plant species. The table includes both hand and honey-bee collected samples, because most samples analyzed have come from honey bee samples. The three species richest in ether extractable material are *Taraxacum officinale*, *Juglans nigra*, and *Populus fremontii*. Although it is visited by pollen-collecting honey bees, *Taraxacum officinale* is primarily apomictic (King and Schaal 1990), not requiring any pollination. *Juglans nigra* (Rink et al. 1989) and *Populus fremontii* (Colin and Jones 1980) are wind-pollinated. The three anemophilous Betulaceae species and the two anemophilous Fagaceae analyzed also contain a relatively rich ether extractable fraction. Values for *Pinus* spp., while lower (2–9%),

are higher than some zoophilous species, such as *Eucalyptus* spp., and *Ceanothus* spp. The uncertain precision of values from honey bee-collected samples precludes statistical analyses on all of the published samples, but it seems unlikely that an overall survey of ether extractable fractions would show entomophilous pollens to contain more extracellular lipids than anemophilous pollens. This in no way casts doubt on the well-demonstrated ability of pollen-consuming insects to cue in on pollen-kitt lipids (Dobson 1987, 1994) or the role of pollen-kitt in attaching pollen to pollinators (Hesse 1979a, b, c); it only casts doubt on the likelihood of either of these specific functions generating an overall association between pollination mode and pollen-kitt abundance.

Species analyzed two or more times generally show similar estimates [e.g. *Phoenix dactylifera* (2.2–3.7%), *Trifolium repens* (2.7–3.8%), and *Zea mays* (2.5–6.7%)]. Estimates for *Typha latifolia* (1.4–7.6) and *Populus fremontii* (6.0–17.8) ranged more widely and, as expected, the estimate for hand-collected *Populus fremontii* pollen exceeded the estimate for honey bee collected pollen.

Pollen-kitt lipids may comprise a small fraction of total pollen lipids. Ibrahim (1974) found that petroleum ether extracted only 1.6% of *Pinus taeda* pollen contents when whole pollen was placed in the solvent. After the pollen was ground in a mill, thus exposing internal pollen lipids to solvent, petroleum ether extracted 6.1% of its material. Similarly, Evans et al. (1991) found that vortexing the intact pollen of *Brassica napus* in solvent yielded only 27.1% of the total lipids extracted when pollen walls had been fractured prior to vortexing. Estimating the total lipids available to pollen-feeding animals will require many more analyses that rupture the pollen walls before extracting the lipids in solvent.

Lipid digestion

Few insects have been studied for their pollen lipid digestion. Pollen lipids were completely digested by larvae of the solitary, specialist bee

Table 2. Percent ether extractable materials in dry pollen samples. Superscripted numbers indicate source of data. Superscripted letters indicate hand (H) or honey bee (B) collected samples

	%
Gymnosperms	
Cupressaceae	
<i>Juniperus utahensis</i>	3.2 ^{1,H}
Pinaceae	
<i>Pinus contorta</i>	2.2 ^{2,B}
<i>P. montana</i>	7.1 ^{3,H}
<i>P. radiata</i>	2.0 ^{2,H}
<i>P. sabiniana</i>	3.2 ^{2,H}
Angiosperms	
Dicots	
Aceraceae	
<i>Acer pseudoplatanus</i>	3.6 ^{4,B}
Actinidiaceae	
<i>Actinidia deliciosa</i> ♀	6.9 ^{5,H}
<i>A. deliciosa</i> ♂	1.9 ^{5,H}
Apiaceae	
<i>Lomatium grayei</i>	8.3 ^{1,B}
Asteraceae	
<i>Agoseris glauca</i>	15.4 ^{1,B}
<i>Centaurea solstitialis</i>	7.8 ^{2,B}
<i>Cichorium intybus</i>	9.5 ^{1,B}
<i>Grindelia squarrosa</i>	7.1 ^{1,B}
<i>Silybum marianum</i>	9.0 ^{2,B}
<i>Taraxacum officinale</i>	18.9 ^{1,B}
<i>T. vulgare</i>	16.2 ^{2,B}
Betulaceae	
<i>Alnus glutinosa</i>	9.4 ^{3,H}
<i>A. incana</i>	13.2 ^{3,H}
<i>Corylus avellana</i>	10.0 ^{6,H}
Brassicaceae	
<i>Brassica campestris</i>	10.7 ^{2,B}
<i>B. kaber</i>	14.5 ^{2,B}
<i>B. nigra</i>	9.9 ^{2,B}
Brassicaceae sp.	7.8 ^{4,B}
Chenopodiaceae	
<i>Salsola kali</i>	7.1 ^{1,B}
Clusiaceae	
<i>Hypericum perforatum</i>	3.2 ^{2,B}
Ericaceae sp.	1.7 ^{4,B}
Fabaceae	
<i>Medicago sativa</i>	8.5 ^{1,B}
<i>Melilotus</i> sp.	8.5 ^{1,B}
<i>Robinia pseudoacacia</i>	12.1 ^{1,B}
<i>Trifolium alexandrinum</i>	6.1 ^{7,B}
<i>T. pratense</i>	14.4 ^{1,B}
<i>T. repens</i>	2.7 ^{4,B} , 3.8 ^{2,B}
<i>Trifolium</i> sp.	3.7 ^{2,B}
<i>Vicia faba</i>	4.0 ^{7,B}
Fabaceae sp.	3.2 ^{2,B}
Fagaceae	
<i>Fagus sylvatica</i>	11.8 ^{4,B}
<i>Quercus kelloggii</i>	7.4 ^{2,H}

Table 2 (continued)

	%
Juglandaceae	
<i>Juglans nigra</i>	18.3 ^{2,H}
Myrtaceae	
<i>Eucalyptus calophylla</i>	1.1 ^{8,B}
<i>E. globulus</i>	1.5 ^{2,B}
<i>E. marginata</i>	0.8 ^{8,B}
<i>Eucalyptus</i> spp.	2.3 ^{7,B}
Oleaceae	
<i>Olea europaea</i>	5.2 ^{2,B}
Onagraceae	
<i>Epilobium angustifolium</i>	2.0 ^{4,B}
Polygonaceae	
<i>Polygonum</i> sp.	1.5 ^{1,B}
Portulacaceae	
<i>Calandrinia ciliata</i>	6.2 ^{2,B}
Ranunculaceae	
<i>Ranunculus arvensis</i>	5.7 ^{1,B}
<i>Ranunculus</i> sp.	2.3 ^{4,B}
Rhamnaceae	
<i>Ceanothus crassifolius</i>	1.3 ^{2,B}
<i>C. integrissimus</i>	1.0 ^{2,B}
Rosaceae	
<i>Chamaebatia foliolosa</i>	5.0 ^{2,B}
<i>Malus pumila</i>	10.4 ^{1,B}
<i>Prunus communis</i>	3.5 ^{2,B}
<i>P. serotina</i>	10.7 ^{1,B}
<i>P. persica</i>	3.0 ^{2,B}
Salicaceae	
<i>Populus fremontii</i>	6.0 ^{1,B} , 17.8 ^{1,H}
<i>P. nigra italica</i>	8.9 ^{1,H}
<i>Salix nigra</i>	4.7 ^{2,B}
<i>Salix</i> sp.	6.1 ^{2,B}
Monocots	
Arecaceae	
<i>Phoenix dactylifera</i>	2.2 ^{7,B} , 3.7 ^{2,B}
Asparagaceae	
<i>Asparagus officinalis</i>	4.5 ^{2,B}
Poaceae	
<i>Cynodon dactylon</i>	2.7 ^{2,B}
<i>Zea mays</i>	2.5 ^{1,B} , 3.9 ⁹ , 6.7 ^{7,B} , 1.8 ^{3,H} , 5.3 ^{3,H} , 3.9 ^{2,H}
Typhaceae	
<i>Typha latifolia</i>	1.4 ^{2,H} , 7.6 ⁹

¹ Standifer (1966)² Todd and Bretherick (1942) (%lipid – %water)³ Nielsen (1955)⁴ McLellan (1977)⁵ Clark and Lintas (1992)⁶ Sosa and Sosa-Bourdouil, in Barbier (1970)⁷ Ibrahim (1974)⁸ Bell et al. 1983 (1983)⁹ Gunasekaran and Andersen 1973, in Stanley and Linskens (1974)

Chelostoma florissomne (Dobson and Peng 1997), but incompletely digested by larval *Osmia* spp. (Suárez-Cervera et al. 1994) and adult honey bees (Peng et al. 1985).

Sterol content

Unlike vertebrates, which can synthesize sterols, insects and other arthropods require dietary sterols for the production of certain hormones, such as ecdysone (Blum 1985). For exclusively herbivorous pollen-feeding arthropods, such as most bee species, dietary sterols come from pollen. Most pollens contain sterols, generally < 1% by dry weight (Stanley and Linskens 1974). The particular sterols determined from the few pollens extensively analyzed are described by Barbier (1970) and Stanley and Linskens (1974). Too few pollens have been analyzed for sterols to compare overall taxonomic or ecological associations with sterol abundance. The most important sterols for honey bees appear to be cholesterol and 24-methylene cholesterol (Svoboda et al. 1980). For details of sterol chemistry in honey bees, see Svoboda et al. (1980, 1982, 1987) and Herbert (1992). Sterols may also serve as pollinator attractants. Hügel (1962) reported that honey bees were attracted to pollen sterols, but Herbert (1992) reported that adding sterols to artificial diet did not increase the diet's attractiveness to honey bees.

Vitamin content

Pollens are rich in water soluble and poor in fat soluble vitamins. Barbier (1970) compiled the known concentrations of vitamins ($\mu\text{g/g}$) in pollens: thiamine (1.4–10.8); riboflavin (5.6–19.2); nicotinic acid (40.7–210); pyridoxine (3.1–9); pantothenic acid (4.2–51); biotin (0.25–0.69); folic acid (3.4–6.8); inositol (3–30 mg/g); ascorbic acid (152–640); vitamin A (0); vitamin D (0.2–0.6); vitamin E (0–0.32); vitamin K (0). Nielsen et al. (1955) reported that the pollen vitamin concentration of the gymnosperm *Pinus montana* generally equalled that of the angiosperms *Zea mays*, *Alnus incana*,

and *Alnus glutinosa*. Herbert (1992) reviewed studies of the dietary vitamin requirements of honey bees. Although synthetic vitamin-deficient diets have been shown to hinder hypopharyngeal gland development and brood rearing, we do not know if differences in vitamin concentration of natural pollens influence development of any pollen-feeding animal.

Caloric value of pollen

The caloric value of pollen, based on bomb calorimetry, ranges from 3635 cal/g for *Broussonetia papyrifera* (Moraceae) to 6750 cal/g for *Ambrosia chamissonis* (Asteraceae) for the set of 100 species analyzed from hand-collected samples (Colin and Jones 1980, Petanidou and Vokou 1990, Shah 1997; excluding honey bee-collected pollens analyzed by Loper and Cohen 1982). Petanidou and Vokou (1990) stated that entomophilous pollens were more calorie-rich than anemophilous pollens, a conclusion not shared by Colin and Jones (1980) or Shah (1997). Petanidou and Vokou (1990) hypothesized that calorie-rich pollen was a plant adaptation to reward insect pollinators. Importantly, four of Petanidou and Vokou's nine most calorie-rich pollens were entomophilous pollens of the Asteraceae, whereas five of the six most calorie-rich pollens in Colin and Jones (1980) data set were anemophilous pollens from the Asteraceae. This clearly underscores the critical importance of phylogeny and the need to sample and compare closely-related taxa that differ in pollination mode. The three studies of caloric content of hand-collected pollen include four plant families from which researchers sampled both anemophilous and entomophilous species. For two of those families, the average caloric content of anemophilous pollen is greater (Asteraceae and Plantaginaceae), whereas for the remaining two families the average entomophilous value is greater (Salicaceae and Anacardiaceae).

Bomb calorimetry values of pollen grains do not correspond simply to digestible calories because they include calories attributable to

poorly digestible portions of the pollen grain, such as the pollen wall. The percent of calories could vary substantially due to differences in pollen surface-to-volume ratios of different sized and shaped grains (Simpson and Neff 1983). We know of no previous calculations, however, that associate pollen wall material with pollen grain size. Cellulose (1–10%) and sporopollenin (4–22%), two main cell wall components, account for a total of 7–23% of the weight of pollen grains (Kwiatkowski and Lubliner-Mianowska 1957). Shah (1997) took a novel approach to subtracting the caloric value of indigestible material from his estimates of caloric values of pollen grains. He acetolyzed the pollen grains of 18 angiosperms and compared the bomb calorimetry values of acetolyzed and unacetolyzed pollen for each species. Because acetolysis removes all of the pollen grain except the sporopollenin-rich exine, he was able to estimate the caloric value of sporopollenin-free pollen. Based on Shah's data, we calculate that the pollen calories attributable to sporopollenin ranged from 4–42% of the total calories per unit weight (whole pollen caloric value correlated with sporopollenin-free pollen, $r = 0.8$). Variation in percent calories due to sporopollenin was not associated with pollen grain diameter ($r^2 = 0.005$, $n = 17$, after removing one outlier), in contrast to Simpson and Neff's (1983) prediction that smaller grains would contain a greater proportion of cell wall material. Exine thickness has been shown to increase with pollen diameter (Lee 1978), which could at least partially negate the effect of decreased surface area of larger grains in terms of digestible calories per unit weight. Exine thickness does not always increase with pollen volume. Grass pollens are often very large but possess very thin exines.

Some adaptive hypotheses have been invoked to predict that entomophilous plants reward or attract their pollinators with calorie-rich pollen (Petanidou and Vokou 1990) or lipid-rich pollens (as estimated by ether extraction, see section on Lipids). Both arguments fail to account for the most calorie-rich

or lipid-rich pollens being anemophilous. These two hypotheses may be closely related, however. Not only would a thicker layer of pollenkit increase a pollen grains' ether-extractable fraction, it would also increase its caloric value. The three plant families producing the most calorie-rich pollens are Asteraceae, Agavaceae, and Juglandaceae. Pollen of the Asteraceae and Juglandaceae, plus the Salicaceae, also yield the most ether-extractable material. The pollen of very few individual species has been analyzed for both caloric value and ether-extractable fraction. Various plant families, however, contain species analyzed for one or the other of the two traits. We have taken these published species values, averaged them across each of 17 plant families, and plotted the average number of calories per family against the average percent ether-extractable fraction (Fig. 1). Many of the species included in the ether-extractable portion have been analyzed only from pollen loads of honey bees. They were included because of the paucity of analyses using hand-collected pollens. Arbitrarily increasing the estimates from honey bee-collected samples to account for added sugar by 20% would lower the r^2 to 0.59, whereas increasing the estimate by 40% would increase the r^2 to 0.80. Despite this imprecision, it is apparent that pollen calorie content is strongly associated with the amount of pollenkit. An unequivocal evolutionary explanation has yet to materialize for different quantities of pollenkit lipids being produced on pollen of different plant species, although these lipids can serve to attract pollinators (Dobson 1988), feed pollinators (Petanidou and Vokou 1990), stick pollen to pollinator bodies (Hesse 1979a, b, c), or perform various other functions (see Pacini 1997).

Water content

Pollen generally contains less than 20% water, but some grasses may contain 50% or more water at the time of dispersal (Stanley and Linskens 1974, Pacini, this volume). Variation in water content among pollens may offset or

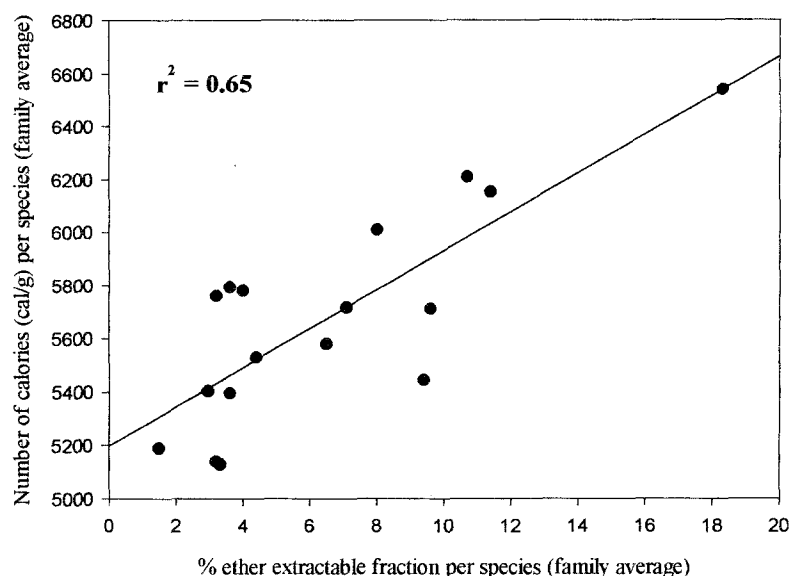


Fig. 1. Caloric value of pollen (cal/g) per plant family plotted against %ether extractable material per plant family. $p < 0.001$

accentuate differences in nutrient content that have been assayed on a dry weight basis. Unfortunately, water content has been estimated for very few pollens, and presently we do not know how it varies with pollen nutrient content.

Toxic pollen?

Various authors have cited studies that reputedly demonstrate that certain pollens are toxic to bees, particularly honey bees (reviewed in Wcislo and Cane, 1996). Suspected pollens come from species of *Aconitum*, *Aesculus*, *Digitalis*, *Ranunculus* (O'Neal and Waller 1984), *Andromeda*, *Astragalus*, *Corynocarpus*, *Hyoscyamus*, *Polygonum*, *Rhododendron*, *Scolypoda*, *Tilia* and *Veratrum* (Stanley and Linskens 1974). In some cases, although subsequent authors cite the toxin source as being pollen, the original studies report extracting whole stamens or even florets, and not just pollen. Other studies [e.g. *Astragalus* (Vansell 1934); *Veratrum* (Vansell 1933)] failed to distinguish the effect of nectar from the effect of pollen in poisoning foragers or brood. Toxic nectars are well known among plants and it has been suggested that they serve to promote pollinator constancy by reducing the visitation of some animal groups, such as lepidopterans,

thus providing all of the floral reward for other groups, such as bees (reviewed in Rhoades and Bergdahl 1981). Hitchcock (1959) shook pollen from the flowers of *Zigadenus venenosus* into sugar water and killed 89% of the bees that fed on it within 16 hours. Thus, it appeared that the toxins, probably alkaloids (Tepedino 1981), were sufficiently abundant in pollen to have a toxic effect. Theoretically, toxins in pollen could guard against pollen foraging by non-pollinators. *Zigadenus venenosus*, however, is apparently bee-pollinated (Moldenke 1976). Tepedino (1989) reported that three bee species of the genus *Dialictus* as well as the putative *Zigadenus* specialist *Andrena astragali*, collected pollen from *Zigadenus nuttallii*. Successful development of any of these bees on *Zigadenus* pollen, however, has not yet been observed. No specialist bee species (reviewed in Wcislo and Cane 1996) have been shown to digest pollens that cannot be digested by generalist bee species.

Toxic substances in some pollens include mannose sugars (Crane 1977, 1978), various alkaloids (Detzel and Wink 1993), and polyphenolics (Carisey and Baucé 1997). Defensive compounds in pollen, when present, are often similar in composition but more dilute than those present in leaves of the same species. Carisey and Baucé (1997) found that concen-

trations of total phenolics and tannins were similar between pollen and foliage of *Abies balsamea*, but that monoterpenes were greatly diluted in pollen. Detzel and Wink (1993) surveyed pollen alkaloid content of *Atropa belladonna*, *Lupinus polyphyllus*, *Brugmansia aurea*, and *Nicotiana tabacum*. All contained alkaloids, but in greatly diminished quantities relative to leaf tissue (*Atropa belladonna* was not analyzed for leaf alkaloids). Nectar contained lesser quantities of alkaloids than pollen in two of the three species surveyed for both. Although Detzel and Wink (1993) carried out alkaloid repellency and toxicity studies with honey bees, it is unclear whether or not the alkaloid concentrations present in all species except *Atropa belladonna* (which contained greater quantities of pollen alkaloids) would repel or poison foraging honey bees. Roulston and Cane (submitted) fed the pollen of *Brugmansia candida* to laboratory colonies of the generalist sweat bee *Lasioglossum zephyrum*. The bees collected and consumed the pollen readily. Their progeny attained as large or larger average body size when fed just *B. candida* pollen than on any of seven other pollens, reflecting this pollen's rich protein content (Roulston et al., in press).

Suggestions for future research

The literature on pollen digestion contains more variables (species examined and pollen characteristics) than equations (experimental observations), which makes it difficult to discern generalities from idiosyncracies. Two specific pollen characteristics have been hypothesized to influence digestibility: pollen wall (both entire and exine) thickness and presence and number of germination pores. Feeding trials with one organism using pollens that differ in these characteristics will help move the study of pollen digestion from a field of opportunistic observations to one of hypothesis testing.

The peculiar specialized "pollen tooth" found on the mandibles of some beetles may represent a rare morphological adaptation associated with pollen mastication. However,

this putative mechanical function has not been satisfactorily demonstrated, so we can not yet conclude that this morphological correlation with pollen-feeding has indeed arisen in order to overcome the resistant pollen wall.

Pollen pellets taken from foraging honey bees are frequently used for chemical analyses of pollen. However, because nectar sugars can add substantial weight to these pellets, they affect all calculations for concentrations of pollen constituents. We lack a reliable laboratory method for removing these nectar sugars from pollen pellets without inadvertently extracting pollen constituents. No study has ever specifically determined the ratios of pollen to nectar-derived sugars in these pellets. We know neither the variability of this ratio, nor what factors control the proportion. Understanding such factors, which are mostly behavioral, will provide insights into the reliability of using these pollen pellets collected by honey bees to quantify pollen constituents. Until these vexing analytical problems are resolved, we must cautiously interpret studies of pollen chemistry that employ as their starting material either harvested whole stamens of flowers or pollen pellets taken from foraging social bees.

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Addresses of the authors: T'ai H. Roulston (e-mail: tai_roulston@NCSU.edu), Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695-7613, USA. James H. Cane, USDA-ARS Bee Biology and Systematics Lab., Utah State University, Logan, UT 84322, USA.