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A phylogenetic reconsideration of the pollen starch–pollination correlation

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ABSTRACT

Mature pollen possesses or lacks starch grains. Many wind-pollinated and self-pollinated species possess starch grains. This apparent association has led some researchers to hypothesize that starchiness of pollen is functionally related to pollen consumption by pollinators. This hypothesis assumes that starchy pollens contain less oil than starchless pollens, and predicts that pollen-consuming insects will be deterred by starchy pollens because of an inability to digest starch or attraction to more oily pollens due to a greater caloric reward. We assembled a database of 207 plant species for which starchiness of pollen and pollination mode are known. We organized these species into a phylogeny based on published cladograms and tested three hypotheses concerning the concordance of pollen starch and pollination mode. We found that starchiness of pollen arose at least 19 times among the 207 species. Using the concentrated changes test, we found that starchiness of pollen did not arise unusually often in wind-pollinated lineages. We found that shifts to starchy pollen did not result in an unusual number of shifts away from bee, fly or beetle pollination. Furthermore, shifts to starchy pollen did not result in an unusual number of shifts away from pollen consumption. There is little evidence to show a general trend of starch avoidance by pollen-consuming insects. Of the three plant species containing the greatest concentration of pollen starch, among the 89 species for which starch has been quantified, corn (*Zea mays*) and English plantain (*Plantago lanceolata*) are readily collected by bees, and cattail (*Typha latifolia*) is commonly used in the artificial rearing of bees. Recent evidence suggests that pollen starch may relate to resistance to desiccation.

Keywords: phylogeny, pollen, pollination, pollination syndrome, starch.

INTRODUCTION

Many floral features have apparently evolved to ensure pollination. Among animal-pollinated species, modified floral traits serve to attract pollinators from a distance, influence pollinator behaviour at flowers, and reward pollinators to ensure return visits or future visits to conspecific individuals. Often, plant species pollinated by the same vectors share many derived floral traits. These suites of derived floral traits have been termed 'pollinator syndromes' (Baker, 1961; Van der Pijl, 1961; Baker and Hurd, 1968).

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One putative pollination syndrome trait is the presence or absence of starch in pollen grains. Calvino (1952) and Baker and Baker (1979) surveyed about 1000 plant species each for presence or absence of pollen starch and reported that more anemophilous (wind-pollinated) than entomophilous (insect-pollinated) species produced starchy pollen. Baker and Baker (1979, 1982, 1983) hypothesized that pollen must store sufficient energy to support pollen tube growth through the style and that this energy was stored primarily as starch or oil. Because starch is biochemically simpler to synthesize and metabolize than oil, Baker and Baker (1979) reasoned that plants should generally use starch as an energy reserve. They predicted that oil would be the primary storage compound in (1) species that possessed small pollen grains and long styles, which necessitated that more energy be stored in a small area, and (2) species pollinated by pollen-feeding insects.

In this paper, we focus on the second of these hypotheses, which suggests that evolutionary shifts towards or away from pollen starch result from evolutionary shifts away from or towards pollination by pollen-feeding vectors. Starchy pollen, according to Baker and Baker (1979), contains less oil than starchless pollen, which makes it an inferior caloric reward. Baker and Baker (1979, 1982, 1983) reasoned that pollen-feeding insects such as bees and some flies, therefore, should seek starchless pollen among potential host plants. Plants pollinated by pollen-feeding insects would be selected to provide the superior reward of starchless pollen. Plants pollinated by other vectors, such as wind, birds and moths, would be selected to provide less rewarding starchy pollen to reduce pollen theft by non-pollinating pollen feeders. Baker and Baker (1979) concluded that there was a statistical association between pollen starch and pollination by non-pollen-feeding vectors after scoring pollen as categorically starchy or starchless for the 1000 plant species in their data set. Statistically, they considered every species to be independent evidence for this association.

We consider the evidence for these hypothetical selection pressures and statistically re-evaluate the conclusion that plant species pollinated by non-pollen-feeding animals are more likely to possess starchy pollen. We look for evidence that starch and oil content of pollen grains are negatively correlated and look for evidence that pollen-feeding animals avoid starchy pollen. Baker and Baker (1979) did not publish their original data set, so we are unable to reconsider their hypothesis with their data. Instead, we created a second survey of about 1000 plant species by combining the extensive categorical starch survey of Franchi *et al*. (1996) with our own small quantitative survey. For all species in our data set, we sought pollination data and reports of pollen feeding by insect visitors. Finally, we searched for published cladograms that included the plant families (resolved to species in some cases, to families in others) in our data set. This process resulted in one overall cladogram of 207 species on which we plotted starch and pollination data. We then compared the distribution of starchiness of pollen with pollination and pollen feeding using the cladogram-based 'concentrated changes' statistic (Maddison, 1990).

METHODS

Starch analyses

Most starch determinations were taken directly from Franchi *et al*. (1996), who published data for 901 plant species. Although Franchi *et al.* placed starchy pollen grains into one of six starch categories based on colour after iodine staining and appearance in polarized light, we have reduced all observations to the categorical distinctions of starchy and starchless.

Baker and Baker (1979) and Calvino (1952) also generated large data sets but published primarily summary data. We sought starch data for individual plant species and did not infer pollen starch status based on surveys of congeners, despite apparent conservation of the trait within certain groups (e.g. Asteraceae, starchless; Poaceae, starchy). Franchi *et al*. (1996) showed pollen starch to be more variable than was apparent previously, including variation within samples and among individuals of the same species in some cases. We scored species shown to be variable by Franchi *et al*. as starchy if individual flowers contained 50% or more starchy grains. For species reported to have starchy and starchless individuals, we scored the trait as mixed.

In addition to gleaning categorical starch data from the literature, we included quantitative starch data for 89 plant species. We used 28 species from Todd and Bretherick (1942) after adjusting their starch estimates by subtracting the weight of water and the weight of reducing sugars from their estimates. The reducing sugars in their analyses appear to derive mainly from the addition of nectar to honey bee pollen loads, the pollen source for their analyses. In previous research, we also adjusted the protein estimates of Todd and Bretherick's (1942) pollen samples by subtracting the entire weight of reducing sugars. Five species analysed by Todd and Bretherick were also analysed by other researchers who used hand-collected pollens. Subtracting the entire weight of the reducing sugars in Todd and Bretherick's samples increased the correlation coefficient between the two data sets from 0.65 to 0.87 (T.H. Roulston, unpublished data).

We also quantified the starch content of 62 species from hand-collected pollen samples. Pollen samples of 10–30 mg were transferred into 13×100 mm test tubes. Five millilitres of 80% ethanol were added and shaken and sonicated several times over 20 min. Five millilitres of 80% ethanol were added and the tubes capped air-tight. The supernatants were removed after gravimetric sedimentation. The ethanol insoluble residue was then washed with 5 ml of 80% ethanol and centrifuged at about 1000 $rev \cdot min^{-1}$. Washing and centrifuging were repeated three more times. The supernatants were discarded and the remaining residue was left to dry overnight. The air-dried pollen residues were reground with a glass rod and treated with 0.6 ml 0.5 N NaOH and vortexed. Then, 2.4 ml of distilled water was added and shaken in a hot water bath (70–80°C) for a few seconds and vortexed several times to finely homogenize the residual starches. The alkaline suspension was then neutralized with 1 ml of 0.33 N acetic acid before the addition of 3 ml of 3 $mg \cdot ml^{-1}$ α-amyloglucozidase enzyme in 0.2 M acetate buffer (pH 4.6). The mixture was incubated in a water bath at 45°C for at least 3 but no more than 5 h. The samples were immersed into a boiling water bath at 97°C for 5 min, cooled and capped, and stored in the refrigerator until analysis. After enzymatic hydrolysis of the pollen samples, an aliquot of 250–1000 μl was reacted with 5 ml *O*-toluidine or *O*-ethyl aniline to determine the glucose content of the starches.

We converted the continuous starch estimates of these 89 species into categorical determinations by considering every estimate greater than 2.5% starch as constituting a 'starchy pollen'. This roughly matched the cut-off point of starchy and starchless species when we compared the percent starch in species quantitatively sampled with categorical determinations made on the same species by previous authors.

Pollination determinations

We systematically searched for pollinator reports for each of the approximately 1000 species for which starch had been determined. We sought reports both on pollen vector and on the

behaviour of animal visitors at flowers (i.e. whether they consumed pollen or not). Because many investigators reporting pollinators or visitors did not report the animals' behaviour on flowers, our scoring of plant species hosting pollen consumers surely underestimates the actual frequency. We searched the CAB Abstracts database for primary literature published between 1973 and 1998. Additionally, we searched several books containing plant–pollinator information on many species: Müller (1883), Robertson (1929), Faegri and Van der Pijl (1979), Free (1993), Roubik (1995) and Proctor *et al*. (1996). Plant species for which pollinators were designated simply as 'insects' in the literature were placed in the 'bees, flies and beetles' category for statistical analyses.

Phylogeny

After we generated a database containing species with both starch and pollination data, we built a cladogram that showed the approximate relationships among sampled taxa. The higher-level phylogeny was based on Chase *et al*. (1993) (Fig. 1a–d). We derived intra-familial and intra-generic relationships from more narrowly focused phylogenetic studies. Focused taxa included Asteraceae (Bayer and Starr, 1998), Caryophyllales (Downie *et al.*, 1997), *Citrus* (Federici *et al.*, 1998), Fabaceae (Kass and Wink, 1995), Lamiaceae (Wagstaff and Olmstead, 1997), Malvaceae (La Duke and Doebley, 1995), Malvales (Judd and Manchester, 1997), Poaceae (Catalan *et al.*, 1997; Soreng and Davis, 1998), *Prunus* (Badenes and Parfitt, 1995), Ranunculaceae (Ro *et al.*, 1997), Rosaceae (Morgan *et al.*, 1994) and Solanaceae (Olmstead and Palmer, 1992). Species that could not be arranged in a completely bifurcating topology based on published literature remained temporarily as polytomies (see below).

Statistical analysis

Concordance of evolutionary changes between pollen starch and pollination mode were tested using the concentrated changes test of the computer program MaClade (Maddison, 1990). The concentrated changes test determines the probability of a character state of one trait being associated with a particular character state of a second trait given the overall distribution of character states on a phylogenetic tree. Thus, it considers both the number of independent origins of character states and their distribution across a tree (Maddison, 1990). Both ACCTRAN and DELTRAN routines within MaClade were used to map the origin of equivocal character state transitions. Probabilities were derived from 1000 simulations.

The concentrated changes test requires the phylogenetic tree to be completely resolved (i.e. lack polytomies). The original tree based on published literature contained 23 polytomies including 3–9 taxa each. Polytomies including only taxa that did not vary in pollination mode or starchiness of pollen were resolved into bifurcating branches arbitrarily. Remaining polytomies were resolved by minimizing apparent homoplasy in the pollen starch character and, when necessary, by minimizing apparent homoplasy in pollinator mode. This approach makes the statistical test more conservative by reducing the number of independent origins of the character states being tested. The concentrated changes test was used to resolve three questions:

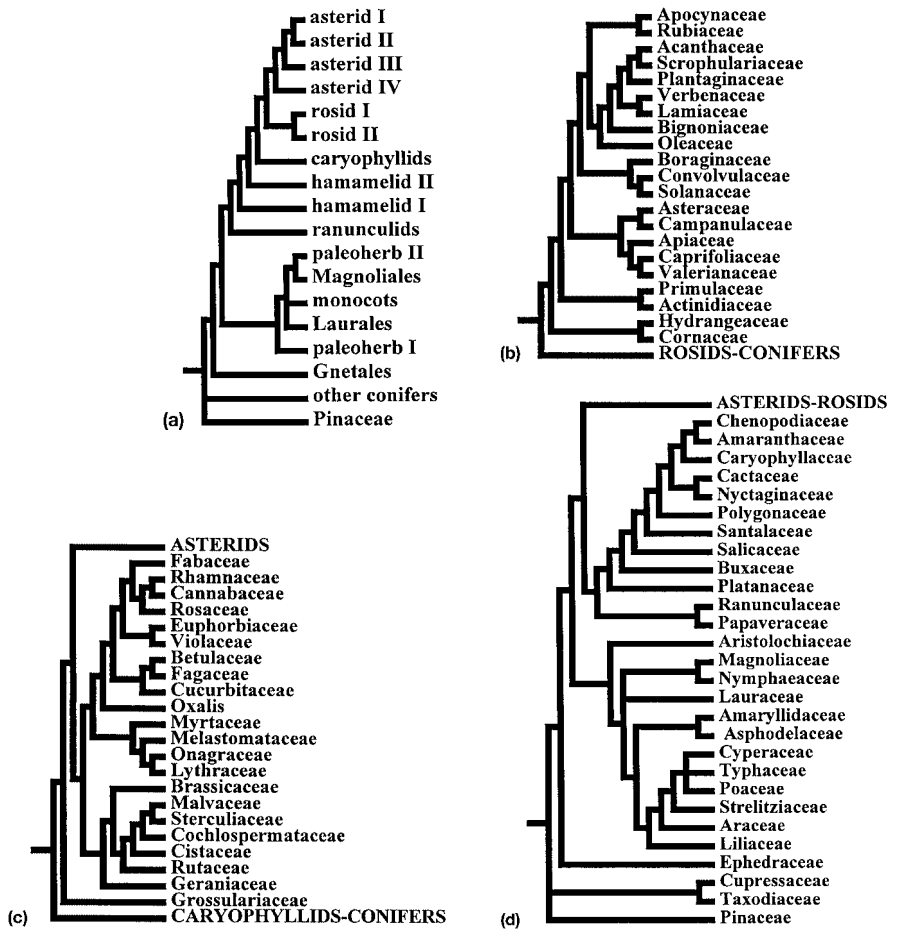

Fig. 1. Arrangement of the gymnosperm and angiosperm clades included in the present survey based on Chase *et al*. (1993): (a) major groups, (b) families of the asterids, (c) families of the rosids, (d) families of the caryophyllids through conifers.

- Do shifts to wind pollination result in shifts to starchy pollen?
- Do shifts to starchy pollen result in shifts away from bee, fly and beetle pollination?
- Do shifts to starchy pollen result in shifts away from pollen consumption by insects?

Percent starch concentration was also compared as a continuous variable between taxa pollinated by bees, beetles and flies and taxa pollinated by other pollen vectors in phylogenetically paired lineages. The 89 species for which starch has been quantified were arranged in a cladogram based on the larger cladogram of 'starchy' and 'starchless' species. The smaller cladogram was then searched for phylogenetic comparisons in which one lineage contained only surveyed species pollinated by bees, beetles and flies, and the sister lineage contained only surveyed species pollinated by other vectors. Percent starch values were averaged for each clade by averaging from the branch tips back to the node of

the common ancestor. The values for each comparison were included in one overall paired *t*-test. This is a similar approach to that used by Herrera *et al.* (1998), except that we used a parametric *t*-test instead of a randomization algorithm.

RESULTS

The original cladogram, including polytomies and based on published phylogenies, is shown in Fig. 2a–c. Starchiness of pollen arose at least 19 times and was lost at least 15

Fig. 2. Starchy (white) and starchless (black) pollens traced along the partially resolved lineage of 207 plant species. Patterned lines indicate ambiguous character state transitions. Numbers beside species' names indicate pollen vector. 0 = wind, 1 = bee, 2 = 'insects', 3 = moth, 4 = fly, 5 = wasp, 6 = butterfly, 7 = apomictic, 8 = beetle, 9 = bird, 10 = bat, 11 = cleistogamy, 12 = thrips. (a) Asterids I–IV, (b) Caryophyllids – conifers, (c) Rosids I–II.

times within the 207 species included in the present survey. Starchless pollen predominated in most lineages. The results of the concentrated changes test did not differ in statistical outcome between the ACCTRAN and DELTRAN algorithms of character optimization. For convenience, we present only the ACCTRAN results. Starchy pollen did not arise unusually often in wind-pollinated lineages ($P = 0.55$; three gains and four losses). Bee, fly and beetle pollination was not statistically more likely to be lost in starchy lineages ($P = 0.12$; no gains and eight losses in starchy lineages, three gains and nine losses in

starchless lineages). Pollen consumption was not statistically more likely to be lost in starchy lineages ($P = 0.34$; six gains and nine losses in starchy lineages, eight gains and 17 losses in starchless lineages).

Pollen ranged from 0 to 22% starch among the 89 species for which starch has been quantified (Table 1, Fig. 3). Most 'starchy' pollens have little starch. Excluding the pollens with less than 2.5% starch, which we scored as starchless, the median starch value was 6.75%. The two starchiest pollens were *Typha latifolia* (mean = 22.3%) and *Zea mays* (mean = 20.1%). These values differed greatly between samples (or analytical techniques). Our *Typha* and *Zea* values were 30.6% and 16.6% starch, respectively, whereas Todd and Bretherick (1942) recorded 13.9% and 25.6% respectively. The histogram of all starch estimates does not provide a clear break in which to distinguish starchy from starchless pollens unless starchless pollens are considered to be those completely lacking in starch. There is a steep drop in the histogram near 5% starch; the use of such a value, however, would greatly reduce the number of species generally considered to be starchy.

Among species for which pollen starch was quantified, pollen starch concentration did not differ between paired lineages pollinated by bees, beetles and flies versus lineages pollinated by other vectors ($t = 1.01$, $P = 0.37$, $n = 5$, paired t-test). There were only five clear phylogenetic comparisons, however (Fig. 4). Of the two comparisons within the Asteraceae, the wind-pollinated lineage contained more starch in one comparison and the insect-pollinated lineage contained more starch in the other comparison. Many of the starchy lineages in the overall cladogram could not be analysed in paired comparisons because the sister clade used the same pollen vector or used multiple pollen vectors. The surveyed gymnosperms, for instance, exhibited considerable change in starch concentration but no change in pollen vector (wind). The caryophyllids for which pollen starch had

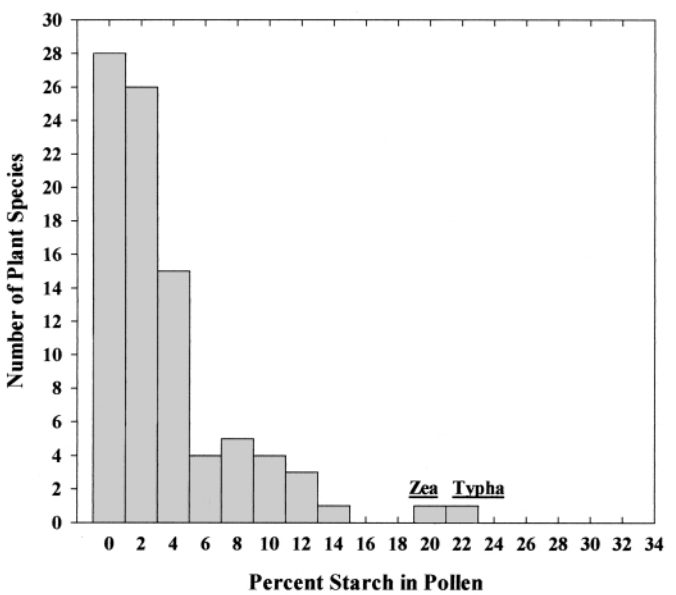

Fig. 3. Histogram of percent starch in pollen grains for 89 plant species.

Table 1. Percent pollen starch content by dry weight of 62 plant species analysed in the present study (not including those of Todd and Bretherick, 1942)

Gymnosperms	
Ephedraceae	
Ephedra trifurca	0.00
Taxodiaceae	
Sequoia sempervierens	2.54
Cupressaceae	
Cupressus macrocaarpa	3.06
Juniperus californica	0.00
Libocedrus decurrens	1.14
Pinaceae	
Cedrus deodora	0.81
Pinus edulis	5.46
Pinus pondersosa	4.00
Anginosperms	
Dicotyledonae	
Aceraceae[b]	
Acer macrophyllum	2.46
Amaranthaceae	
Amaranthus palmeri	7.52
Amaranthus spinosus	1.19
Anacardiaceae[b]	
Schinus molle	5.62
Asteraceae	
Ambrosia bipinnatifida	0.00
Ambrosia dumosa	0.21
Artemisia tridentata	0.54
Chrysanthemum leucanthemum	0.00
Encelia farinosa	0.38
Helianthus annuus	0.39
Iva axillaris	0.00
Taraxacum officinalis	0.00
Brassicaceae	
Brassica campestris[a]	0.00
Cannabaceae	
Cannabis sativa	1.14
Caprifoliaceae	
Sambucus glauca	0.00
Chenopodiaceae	
Chenopodium album	7.41
Cochlospermataceae	
Cochlospermum vitifolium	0.00
Fabaceae	
Cercidium torreyanum	0.32
Fagaceae	
Quercus agrifolia	0.27
Quercus kellogii[a]	0.35
Quercus wislezenii	1.97
Melastomataceae	
Bellucia imperialis	0.00
Papaveraceae	
Eschscholzia californica	0.00
Plantaginaceae	
Plantago lanceolata	11.10
Platanaceae	
Platanus racemosa	0.24
Polygonaceae	
Rumex conglomeratus	10.03
Rumex crispus	9.10
Rumex hymenosepalus	9.34
Primulaceae	
Dodecatheon clevelandii	0.00
Rosaceae	
Adenostoma fasciculatum	2.44
Rosa woodsii	1.70
Salicacea	
Populus fremontii	0.94
Simmondsiacea	
Simmondsia chinensis	0.00
Solanaceae	
Brugmansia candida	0.00
Datura inoxia quinqucuspida	0.00
Solanum douglassii	0.00
Solanum eleagnifolium	0.00
Solanum lycopersicum	0.00
Solanum melongena	0.00
Solanum xanti	0.00
Monocotyledonae	
Agavaceae[b]	
Agave americana	0.00
Agave chrysantha	0.00
Agave schotti	0.00
Arecaceae[b]	
Cocos plumifera	0.00
Phoenix dactylifera	0.00
Asphodelaceae	
Aloe ferox	0.00
Poaceae	
Agropyron smithii	10.88
Bromus carinatus	8.35
Dactylis glomerata	1.70
Holcus lanatus	3.50
Phleum pratense	4.60
Poa annua	6.09
Zea mays	16.56
Typhaceae	
Typha latifolia	30.65

[a] Also surveyed by Todd and Bretherick (1942). [b] Plant families not plotted on overall cladogram due to uncertainty of placement.

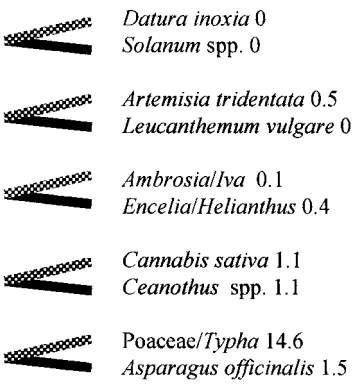

Fig. 4. Comparison of pollen starch concentration in phylogenetically paired lineages pollinated by bees, beetles and flies (black) versus other vectors (chequered).

been quantified (*Amaranthus*, *Chenopodium* and *Rumex*) were all wind-pollinated, which precluded comparisons within the caryophyllids. It is probable that the *Amaranthus–Chenopodium* clade is much starchier than the *Silene–Stellaria–Saponaria* clade (Fig. 2b), given that the latter clade is 'starchless' according to Franchi *et al*. (1996). Assigning the *Silene–Stellaria–Saponaria* clade a value of zero starch and comparing it with the *Amaranthus–Chenopodium* clade (7.43% starch) in the paired comparisons did not change the statistical outcome of the test.

DISCUSSION

Starchiness of pollen does not appear to respond greatly to, or influence, pollination mode on an evolutionary scale. Although the shifts in pollination mode within starchy lineages are biased away from pollinators that consume pollen, shifts in pollination mode are similarly biased in starchless lineages. For instance, bee, beetle and fly pollination was lost eight times and never gained within starchy lineages. If we had considered the null model to be 50% gains and 50% losses, then we would have found statistical evidence that pollen starch biased the direction of shifts in pollination mode. Bee, beetle and fly pollination, however, was lost nine times and gained only three times in starchless lineages. This reveals an evolutionary trend, independent of pollen chemistry, away from pollen-consuming pollinators. This interpretation contradicts those of Calvino (1952) and Baker and Baker (1979). Although these researchers worked with large data sets and discussed the phylogenetic distribution of their data, they did not perform their analyses in a statistically explicit phylogenetic manner. Baker and Baker (1983) used a statistical approach (chi-squared) that treated every sampled species as an independent evolutionary unit and found that anemophilous and autogamous species were significantly more likely to be starchy than plants pollinated by other vectors. They also concluded that nectarless species and hummingbird-pollinated species were significantly more likely to be starchless than plants pollinated by other vectors. Despite finding a smaller percentage of starchy pollens among hummingbird-pollinated species than any other pollination group, they concluded that starchy pollens were typical of bird pollination. They justified this by hypothesizing that the longer styles of bird-pollinated species required the more energy-efficient use of oils

as an energy storage compound so that pollen tubes could reach ovules. Thus, they argued that selection for pollen tube growth reduced pollinator selection on carbohydrate storage compounds.

The use of a simple chi-squared test is inadvertently weighted by multiple sampling within lineages where no changes of character state have occurred (Harvey and Pagel, 1991). The degrees of freedom for the test arises from the total number of species sampled instead of the number of independent evolutionary origins of each character state. Baker and Baker expressed concern for this problem, but neither phylogenies nor statistical approaches based on phylogenies were commonly used when their work was published. The concentrated changes test used in the present study avoids this bias by testing the probability of trait association within a given phylogeny. The concentrated changes test, however, also has biases. When a phylogeny includes only a representative rather than an exhaustive sample of taxa, as in the present study, the test may be sensitive to the number of taxa included in surveyed lineages (Maddison, 1990). We included all species for which both pollination and starch data were available.

In theory, the problem of selective sampling can be avoided by comparing pollination mode and pollen starch within small phylogenetic groups. When character states are highly conserved, however, as with pollen starch, a small phylogenetic group may not show sufficient variation in the traits of interest to reveal evolutionary trends. This is apparent in the only groups intensively sampled for pollen starch. Baker and Baker (1982) examined 17 genera and 76 species and subspecies in the Onagraceae and found starchy pollen in all species except those of the genera *Fuchsia* and *Lopezia*. These genera appear to be sister taxa and arise near the base of the phylogenetic tree for the Onagraceae (Hoch *et al.*, 1993). Based on the cladogram of Hoch *et al*. (1993) and the pollination studies of Raven (1979), the derivation of starchless pollen occurred in lineages pollinated mainly by birds (*Fuchsia*) or birds, flies and bees (*Lopezia*), while numerous shifts between bee, moth, bird, fly and butterfly pollination occurred in lineages that retained the starchy character state (Fig. 5). Thus, there was no support for the hypothesis that pollen starch reflects pollen use by pollinators in the Onagraceae.

Grayum (1985) surveyed the plant family Araceae for pollen starch and also found little support for an association between pollen starch and pollination. The two aroid genera with bee pollination and starchless pollen (*Anthurium* and *Spathiphyllum*) were pollinated by male euglossine bees, which collect scent instead of pollen. The other bee-pollinated genus (*Monstera*) had starchy pollen. Grayum also found no apparent association between pollen starch and fly pollination. Unfortunately, the phylogeny and pollination of the Araceae are too poorly understood to examine the distribution of pollen starch explicitly.

The accuracy of the concentrated changes test, or any other phylogenetic test, depends on the accuracy of the cladogram on which the characters are traced. The overall topology of the cladogram used in this study was derived primarily from Chase *et al*. (1993). Some parts of Chase and co-workers' cladogram conflict with more recent studies. We reconstructed the cladogram by repositioning some of the more troublesome clades. The work of Chaw *et al*. (1997) supports a monophyletic gymnosperm clade that includes a monophyletic Gnetales and a monophyletic Coniferales. Their work also suggests alternate positions for the Nymphaeaceae and the Aristolochiaceae. Repositioning these clades in our test had no influence on the results.

The previously proposed functional relationship between pollen starch and pollination assumes that pollen-consuming insects prefer starchless pollen. This could result from either

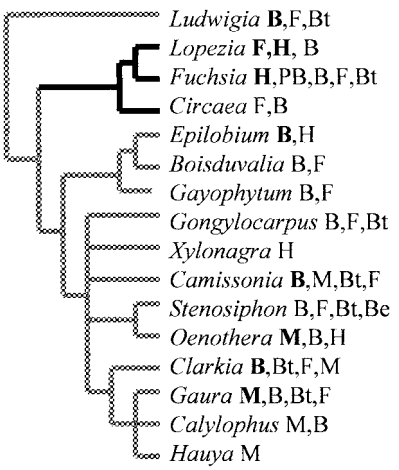

Fig. 5. Starchy (chequered) and starchless (black) pollens traced along the genera of the Onagraceae. Cladogram based on Hoch *et al.* (1993). Pollination modes reported by Raven (1979). Bold letters indicate primary mode of pollination. B = bee, F = fly, H = hummingbird, PB = perching birds, Bt = butterfly, M = moth, Be = beetle.

starch acting as a deterrent or the alternative to starch (speculated to be oil) acting as an attractant to pollinators. The only support for starch acting as a deterrent stems from observations that adult honey bees cannot digest starch (see Schmidt *et al.*, 1989, and references therein). Most recent evidence, however, indicates that honey bees can digest starch readily if it is released from the amyloplasts (Klungness and Peng, 1984). Other bee species can also digest starch and many collect starchy pollens. The solitary bee *Diadasia afflicta* collects pollen only from *Callirhoe* spp. (Malvaceae) and breaks down the starch during digestion (Simpson and Neff, 1983). The solitary bee *Chelostoma florisomne* also collects starchy pollen from its host plant, *Ranunculus* spp., and digests it very efficiently (Dobson and Peng, 1997). *Osmia cornuta* consumes both starchy and starchless pollens. *Osmia cornuta* larvae completely remove the starch from *Euphorbia helioscopia* pollen, but not from *Ranunculus* sp. (Nepi *et al.*, 1997). Many plants with starchy pollen (e.g. the Onagraceae and Malvaceae) are commonly pollinated by bees that collect the pollen (e.g. Kerfoot, 1967; Bohart and Youssef, 1976).

The three starchiest plant species presently known (excluding *Salix* sp., which was not identified to species) are *Typha latifolia* (cattail), *Zea mays* (corn) and *Plantago lanceolata* (English plantain). Cattail and corn are wind-pollinated and plantain is pollinated by wind and bees. Few, if any, bees collect cattail pollen in the wild. Cattail pollen, however, is one of the main pollens used in the artificial rearing of bumble bees (e.g. Heinrich, 1979) and sweat bees (Greenberg, 1982), which readily collect and consume it and survive to adulthood. Adult honey bees, in contrast, do poorly when fed only cattail pollen (Schmidt *et al.*, 1989). Honey bees often collect corn pollen (Mason and Tracewski, 1982; Flottum *et al.*, 1983; Erickson *et al.*, 1988; Schmidt *et al.*, 1989; Malerbo and dos S. Noguiera-Couto, 1992; Vaissiere and Vinson, 1994) and develop healthy hypopharyngeal glands and sufficient body fat while consuming it (Shuel, 1992). Bumble bees (*Bombus* spp.) and various solitary bee species also readily collect corn pollen (personal observation). Plantain pollens (*Plantago*

spp.) are frequently collected by honey bees (Petkova, 1984; Wille *et al.*, 1985) and bumble bees (personal observation).

Baker and Baker's starch hypothesis assumes that pollen grains store starch as an energy reserve in place of oil. They reasoned that because it is metabolically simpler both to produce and metabolize starch than oil, plants should generally use starch instead of oil as a storage compound. Starch has the disadvantage, however, of storing less energy per unit volume than oil. Thus, small pollen grains whose pollen tubes have to grow great distances before reaching an ovule or maternal energy-rich tissues might need to store energy as oil rather than starch. Baker and Baker (1979, 1982) and Grayum (1985) provided support for this idea by reporting that small pollen grains almost never contain starch, whereas large pollen grains commonly contain starch. The idea that pollen grain size relates to energy storage capacity is supported by several studies that found a strong association between pollen grain volume and style length (Lee, 1978; Plitmann and Levin, 1983; Ramamoorthy *et al.*, 1992; Roulston *et al.*, in press) or pollen grain volume and stigma depth (Cruden and Lyon, 1985).

Baker and Baker's hypothesis predicts that internal pollen lipids and pollen starch will be negatively correlated. At present, there are insufficient data to test this prediction. Many plant species have been surveyed for pollen lipids through whole pollen emersion in a solvent such as ether or benzene (e.g. Todd and Bretherick, 1942). Simple emersion, however, may remove primarily external pollen lipids and leave most internal lipids inside pollen grains. Evans *et al.* (1991) compared lipid retrieval from *Brassica napus* pollen that had been pre-treated to fracture the pollen wall with lipid retrieval from intact pollen. They found that intact pollen yielded only 27.1% of the lipids yielded by fractured grains. Scott and Strohl (1962) extracted four times more material from *Pinus taeda* pollen that had been ground in a ball-mill than from intact pollen. Thus, a test of the putative internal pollen lipid–starch trade-off will require as yet ungenerated data sets that quantify internal pollen lipids.

Biochemical observations on the fate of pollen starch reveal that pollen grains may often trade starch for sugars rather than lipids. Immature pollen grains of all species contain starch grains in amyloplasts (Baker and Baker, 1979; Franchi *et al.*, 1996). Some plant species retain starch grains in amyloplasts throughout anther dehiscence and pollen dispersal. Other species hydrolyse pollen starch into cytoplasmic sugars and cytoplasmic carbohydrates (Pacini, 1996). Speranza *et al.* (1997) found that starch content was inversely related to sucrose concentration of pollen grains across a sample of 13 plant species ($r^2 = 0.71$). Speranza *et al.* provided evidence that starch hydrolysis of maturing pollen grains determined the sucrose content of the cytoplasm. The conversion of pollen starch into sucrose helps protect pollen membranes against desiccation in some species (Hoestra and Van Roekel, 1988). Franchi *et al.* (1996) hypothesized that habitat exposure may select for starchless grains because species that live in dry, open environments must convert starch into sucrose to prevent desiccation. They support this hypothesis with observations that plant species from arid environments usually contain starchless pollen and that cleistogamous flowers are often starchy, whereas chasmogamous flowers on the same plant are often starchless.

Pollen starch may vary between seasons or temperature regimes within species. *Antirrhinum tortuosum* growing in Germany produced starchless pollen in summer and starchy pollen in November; *Pelargonium* sp. contained less pollen starch when grown at 25°C than when grown at 15°C (Molisch, 1893; Sears and Metcalf, 1926, cited in Baker and

Baker, 1979). Both of these observations are consistent with the hypothesis that starch content may be functionally related to desiccation. They also show that starch content may be phenotypically plastic. Given the clear functional mechanism on which this hypothesis hinges, it should be possible to test it through viability trials with starchy and starchless grains at various levels of exposure.

Based on available information, we conclude that there is insufficient evidence to confirm an evolutionary trend between pollen starch and pollination mode or pollen starch and pollen consumption. Additionally, we conclude from the literature on bee collection, consumption and digestion of the starchiest pollens, that pollen starch may not deter pollen-collecting bees. Biochemical observations suggest that all pollen is starchy when immature and that starch is frequently converted to sugars, which may help protect pollen grains from desiccation.

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