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POLLEN DIGESTION BY NEW WORLD BATS: EFFECTS OF PROCESSING TIME AND FEEDING HABITS

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Abstract. Although pollen is included in the diet of vertebrates and invertebrates, and extraction efficiency of its contents has been estimated for several flower visitors, the relationship between feeding habits and pollen digestion efficiency has not been carefully studied. We compared the efficiency with which four species of New World bats with different feeding habits extracted the contents of different types of pollen, and we tested the hypothesis that flower-visiting bats have higher extraction efficiencies than fruit-eating bats. We gave doses of different types of pollen to two nectarivorous (*Anoura geoffroyi* and *Leptonycteris curasoae*) and two frugivorous (*Artibeus jamaicensis* and *Sturnira lilium*) bats and collected their feces at regular intervals. We used pollen from three species of flowers that are associated with bat visitation: *Pseudobombax ellipticum, Hylocereus undatus,* and an unidentified species of night-blooming, columnar cactus. In addition to estimating the percentage of empty pollen grains in the feces, we determined digesta time distributions in the gastrointestinal tract and interpreted them using chemical reactor theory. Extraction efficiency was higher in bats that regularly include pollen in their diet. This pattern was not explained by differences in the rate with which the bats processed pollen, or by the time that pollen was retained in the stomach, where little degradation of pollen grains occurs. Within species, however, the percentage of empty grains increased asymptotically with time in the gastrointestinal tract. In general, the digestive system of the bats seemed to process pollen grains as a continuous stirred-tank reactor (CSTR) connected to a plug-flow reactor (PFR), with longitudinal mixing in the PFR: the food was retained for a relatively short period in the stomach and then was moved through the intestine, where it was mixed longitudinally. *Artibeus jamaicensis* was an exception to this pattern in the way its digestive system processed *H. undatus* pollen; in this case, its gastrointestinal tract apparently functioned as a PFR with a considerable amount of longitudinal mixing. We hypothesize that differences among bats in extraction efficiency of pollen contents may be partly explained by differences in the intestinal activity of the enzymes responsible for pollen grain degradation.

Key words: Anoura geoffroyi; Artibeus jamaicensis; *bats, frugivorous and nectarivorous; chemical reactor models; food processing time;* Leptonycteris curasoae; *plant–animal interactions; pollen digestion;* Sturnira lilium.

Introduction

Pollen grains are included in the diet of several vertebrate and invertebrate flower visitors (Howell 1974*a*, Turner 1984, Peng et al. 1986, Richardson et al. 1986, Wooller et al. 1988, Law 1992*a*, *b*, Grant 1996). Although pollen can be an important source of proteins, vitamins, and minerals, its exine coat is highly resistant to degradation by digestive enzymes (Stanley and Linskens 1974). Several studies have estimated the efficiency and rate at which vertebrate digestive systems process pollen grains under experimental and natural conditions (Turner 1984, Richardson et al. 1986, Wooller et al. 1988, Brice et al. 1989). Extraction efficiencies of pollen contents in these studies ranged from 7% to 100%, whereas pollen transit through the gut took from 30 min to several hours.

Comparative studies of pollen digestive processing in animals that have different diets do not yet provide a satisfactory picture of the digestive adaptations of flower visitors. Wooller et al. (1988) concluded that pollen digestion in specialized avian pollen feeders did not differ from the digestive performance of birds that do not include pollen in their natural diets. However, their work did not have a standard methodology for comparing the different species they studied. Some bat species regularly include pollen in their diet (Gardner 1977) and are able to extract its contents (Howell 1974*a*, Law 1992*a*). Because of the broad dietary spectrum showed by its members (Gardner 1977), the fam-

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ily Phyllostomidae offers a unique opportunity to compare pollen extraction efficiencies among animals with different feeding habits. In this study, we compared the efficiency with which phyllostomid bats with different degrees of flower specialization extract the contents of three species of pollen. We hypothesized that bat species that regularly include pollen in their diet should be more efficient at extracting pollen contents than species that ingest pollen only seasonally.

Methods

We conducted this study with nonreproductive adults of four species of phyllostomids: *Anoura geoffroyi* (Glossophaginae), *Leptonycteris curasoae* (Glossophaginae), *Artibeus jamaicensis* (Stenodermatinae), and *Sturnira lilium* (Stenodermatinae). *Artibeus jamaicensis, S. lilium,* and *A. geoffroyi* are species resident to the study site, whereas *L. curasoae* is present only during late spring and summer. *L. curasoae* is considered a derived nectarivorous species (Koopman 1981); its diet includes pollen, nectar, fruit, and a small amount of insects (Gardner 1977). Pollen constitutes an important part of the diet of this species throughout the year (Alvarez and Gonzalez 1970, Fleming 1995, Valiente-Banuet et al. 1996). Fleming et al. (1993) postulate that migratory populations of the subspecies *L. c. yerbabuenae* move north from central Mexico along a nectar corridor of blooming columnar cacti in the spring, and then move south following blooming paniculate agaves in the fall. Members of this subspecies spend the winter and fall in central and southern Mexico, where they feed on nectar and pollen from flowers in the Bombacaceae, Convolvulaceae, Leguminosae, Agavaceae, and Cactaceae. Similarly, nonmigratory populations of *L. c. yerbabuenae* in northern and central Mexico feed on flowers year-round (Fleming 1995). According to Alvarez and González (1970) and Howell (1974*b*), insects account for a very small fraction of the diet of *L. curasoae.*

According to Koopman (1981), *Anoura geoffroyi* is a more primitive and more generalized nectarivore than *L. curasoae*; its diet includes pollen, nectar, fruits, and insects (Gardner 1977). Alvarez and González (1970) considered this species to be a facultative pollen eater. Stomachs and feces of *A. geoffroyi* have been reported to contain pollen as well as large amounts of insect remains (Alvarez and González 1970, Howell 1974*b*, Sazima 1976, Willig et al. 1993).

Artibeus jamaicensis feeds on fruits, insects, pollen, and nectar (Gardner 1977). Heithaus et al. (1975) considered it to be primarily a frugivore that feeds heavily on nectar and pollen during the dry season in tropical dry forests. During the wet season, when flower resources are scarce and fruits are more abundant, this species switches back to a diet of mostly fruit. The importance of fruits with respect to pollen during the dry season, however, is probably underestimated. Pollen consumption was inferred from samples obtained from bat fur, whereas fruit ingestion was determined by examining fecal samples; the absence of fruit remains in most (92%) fecal samples of *A. jamaicensis* probably reflects either short fruit retention times in the gut, or the fact that large, single seeds of some fruits are not ingested (Heithaus et al. 1975). In a central Mexico desert, *A. jamaicensis* feeds occasionally on the pollen and nectar of *Neobuxbaumia tetetzo,* a species of columnar cactus, but it is a much more common visitor to this plant when fruit is available (Valiente-Banuet et al. 1996). Insects are a secondary part of the diet of *A. jamaicensis* (Fleming et al. 1972).

Sturnira lilium feeds largely on fruits, but it also visits flowers and presumably ingests nectar and pollen (Gardner 1977). In common with *A. jamaicensis,* this species apparently shifts from nectarivory in the dry season to frugivory in the wet season in tropical dry forests (Heithaus et al. 1975). Similarly to *A. jamaicensis,* the importance of fruits in the diet of *S. lilium* during the dry season is probably underestimated (Heitahus et al. 1975). This species apparently does not include insects in its diet (Fleming et al. 1972, Willig et al. 1993).

Although feeding habits were not systematically followed in the study site for the species of bats investigated, anecdotal evidence supports the patterns previously described. We never captured individuals of *A. jamaicensis* or *S. lilium* bearing pollen on their body, and we never found pollen grains in the few fecal samples examined for these species. However, we often captured individuals of *L. curasoae* and *A. geoffroyi* with pollen-covered heads and often found pollen grains in their feces.

Animals were captured and experiments were conducted at Orizaba, Veracruz, Mexico (18°51′ N, 97° 05′ W). The site is located at an elevation of 1240 m and has an average annual precipitation of 2035 mm (Gomez-Pompa 1982). Bats were netted in caves, orchards, and old buildings at the study site and were released at the end of the experiments. We estimated the efficiency of digestive processing of pollen grains from *Pseudobombax ellipticum* (Bombacaceae; triangular pollen grains, 70 μm long), *Hylocereus undatus* (Cactaceae; spherical pollen grains, 80 μm diameter), and an unidentified species of columnar cactus with nocturnal flowering (spherical pollen grains, 80 μm diameter). Six individuals of *A. geoffroyi* (four females and two males, body mass 14.4 ± 1.36 g, mean ± 1 SD), *A. jamaicensis* (two females, four males, 42.1 ± 4.06 g), and *S. lilium* (two females, four males, 21.4 ± 0.83 g) were given pollen from *P. ellipticum*; 11 *A. geoffroyi* (six males, five females, 15.0 ± 1.25 g) and seven *A. jamaicensis* (five males, two females, 38.2 ± 2.33 g), were given pollen from *H. undatus*; and five individuals each of *A. geoffroyi* (three females, two males, 14.5 ± 1.27 g), *A. jamaicensis* (one female, four males, 39.9 ± 7.08 g), *L. curasoae* (five males, 23.3 ± 1.78 g), and *S. lilium* (two females, three males, 20.3

± 1.01 g) were given pollen from the columnar cactus. We followed the procedures we will now outline for the three types of pollen.

For three days before each trial, bats were kept in individual, cylindrical wire cages (40 cm diameter × 50 cm high) and were fed mango juice (Jumex) with powdered soya milk (Bonus), corn oil (Maravilla), and ABCDE vitamins and minerals (Nature Made). During this period, the bats were habituated to human presence while they were feeding. On the night of each trial and after it had fed ad libitum on mango juice, we gave each bat an oral dose of known volume (~1 mL) of freshly collected pollen dissolved in distilled water. It has been demonstrated previously that the number of empty grains increases slightly after incubating pollen grains in water during 5 h (Grant 1996). In our study, pollen solutions were administered to bats <30 min after being prepared. Although the percentage of empty grains in the solutions offered to the bats was not systematically determined, we observed no evidence of a reduction in the proportion of full grains with respect to pollen directly collected from the flower.

We used a dosing syringe to administer the solution, and the bat was allowed to continue feeding on mango juice for the rest of the night. We recorded the time when the dose was applied and collected fecal material from the floor and walls of the cage every 15 min for the first two hours and every 30 min for three more hours. Feces collected at each time interval were placed in plastic vials, Alexander's solution was added (Alexander 1969), and the vials were placed in an oven at 60°C for 24 h as soon as they were collected. Pollen from the flowers used in each trial was subjected to the same treatment as feces. Alexander's solution stains empty (''digested'') grains green and full (''undigested'') grains red (Law 1992*a*).

We examined stained feces with a compound microscope and counted the number of empty and full pollen grains in a sample of ≥500 grains whenever possible. To estimate the total number of pollen grains, we examined the total volume of stained feces collected at each time interval by spreading one drop at a time of feces on 4–8 slides over the standard size of a coverslip. We then counted the number of grains in one-third of the total number of fields of view in one coverslip and multiplied by three the total number of grains in the fields examined to obtain the total number of grains in the drop of feces. The number of grains estimated for each drop was then added to obtain the total number of grains at each time interval. The number of drops examined varied according to the total volume of feces collected at each interval (e.g., small volumes of feces required the examination of no more than four drops). We also counted the number of empty and full grains from ≥200 grains in the samples taken from the flowers (2.7, 2.0, and 14.0% empty grains for *P. ellipticum, H. undatus,* and columnar cactus flowers, respectively). We estimated the percentage of emptied grains in the bat's gut by subtracting the percentage of naturally occurring empty grains from the percentage of empty grains in the feces.

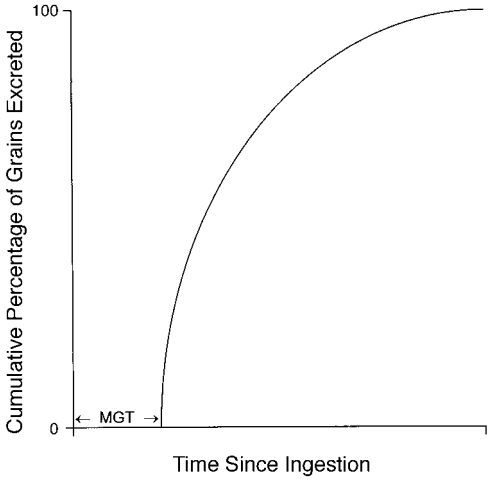

FIG. 1. Expected cumulative excretion curve (*F* curve) of pollen grains for a gastrointestinal tract that behaves as a continuous stirred-tank reactor (CSTR) in series with a plug-flow reactor (PFR). The time between ingestion of the pollen doses and the first appearance of grains in the feces represents minimum gut transit time (MGT) and is equivalent to time spent in the intestine. The difference between gut mean retention time (MRT) and MGT is comparable to mean retention time in the stomach (CSTR) (Martínez del Rio et al. 1994).

We plotted cumulative pollen grain excretion (*F* curves) and interpreted them using chemical reactor models (Penry and Jumars 1987). The gastrointestinal tracts of frugivorous and nectarivorous phyllostomids probably function as a continuous stirred-tank reactor (CSTR) in series with a plug-flow reactor (PFR), analogous to the stomach and the intestine, respectively (Forman et al. 1979, Martínez del Rio et al. 1994). The *F* curve of an ideal CSTR-PFR is an exponential curve shifted to the right of time zero, the time when the dose was given (Fig. 1; Martínez del Rio et al. 1994). We estimated minimum gut transit time (MGT) and mean retention time (MRT) of pollen grains to the nearest minute. MGT is the time elapsed from ingestion of the pollen solution to the first appearance of pollen grains in the feces. MRT is the length of time that the average pollen grain remains in the gut; it was calculated using the equation

$$\text{MRT} = (\Sigma\, m_i t_i)/\, \Sigma\, m_i$$

where m_i is the number of pollen grains excreted at the ith defecation at time t_i after ingesting the pollen dose. In an ideal CSTR-PFR tract, MRT is equal to MGT plus the mean retention time in the stomach (Martínez del Rio et al. 1994). We plotted the natural logarithm of the number of pollen grains excreted against the length time since ingestion, and used the terminal portion of this curve to estimate the slope (k). The inverse of k estimates the retention time in the CSTR (Karasov

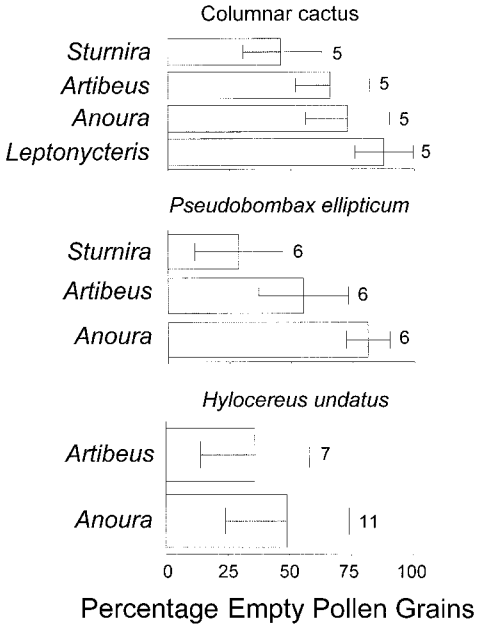

FIG. 2. Mean percentage of empty pollen grains and standard deviations for each species of bat and pollen type. Numbers along the bars represent sample sizes.

and Cork 1996) and was used to determine whether or not the gastrointestinal tract of our bats fits a CSTR-PFR model. We also calculated the degree of longitudinal mixing in the reactor by estimating the vessel dispersion number ($D/\mu L$), using the formula

$$\sigma^2/\text{MRT}^2 = 2(D/\mu L) + 8\ (D/\mu L)^2$$

where

$$\sigma^2 = [\Sigma\ (t_i - \text{MRT})^2 m_i]/\Sigma\ m_i.$$

No mixing is indicated by a $D/\mu L$ value of zero, and the value approaches infinity when there is mixed flow. Values within this range indicate small (0.002), intermediate (0.025), or large amounts (0.200) of longitudinal mixing (Levenspiel 1972).

Because retention time in the gut increases with body mass$^{0.25}$ in birds and other vertebrates (Karasov 1990), we corrected for body mass by dividing MRT and MGT by body mass$^{0.25}$ to conduct interspecific comparisons.

RESULTS

Extraction efficiency and time of gut processing

Pseudobombax ellipticum *pollen digestive processing.*—The percentage of empty pollen grains differed significantly among the three species of bats (Fig. 2; one-way ANOVA: $F = 16.70$, $P = 0.0002$, df = 2, 15). *Anoura geoffroyi* was the most efficient species at emptying pollen grains, followed by *Artibeus jamaicensis* and *Sturnira lilium* (Tukey's hsd test, $P < 0.05$).

Mean gut retention times did not differ significantly among *A. geoffroyi*, *A. jamaicensis*, and *S. lilium* (Fig. 3; one-way ANOVA: $F = 2.54$, $P = 0.118$, df = 2, 15), and minimum gut transit times were significantly different only between *A. jamaicensis and S. lilium* (Fig. 3; log-transformed data, one-way ANOVA: $F = 8.14$, $P = 0.004$, df = 2, 15, Tukey's hsd test, $P < 0.05$). Longer MGTs were detected in *A. jamaicensis*, followed by *A. geoffroyi* and *S. lilium.* No significant differences in MRT and MGT were found among the three species of bats on a mass-specific basis (Fig. 3; one-way ANOVA, MRT: $F = 0.09$, $P = 0.917$, df 2, 15; MGT: $F = 0.49$, $P = 0.623$, df = 2, 15).

Although neither MRT nor MGT was significantly related to the mean percentage of empty grains in any of the species (MRT: *A. geoffroyi*, $r = 0.320$, *A. jamaicensis*, $r = 0.392$ *S. lilium*, $r = 0.447$; MGT: *A. geoffroyi*, $r = 0.453$, *A. jamaicensis*, $r = 0.059$, *S. lilium*, $r = 0.320$; all $P > 0.112$, df = 1, 4), in general, the percentage of empty grains increased asymptotically with time spent in the gut in individuals of the three species (Fig. 4).

Hylocereus undatus *pollen digestive processing.*—There were no significant differences in the percentage of empty grains between *A. geoffroyi* and *A. jamaicensis* ($t = 1.02$, $P = 0.324$, df = 16), although the former species had a higher mean (Fig. 2). Neither MRT ($t = 1.65$, $P = 0.117$, df = 16) nor MGT ($t = 1.58$, $P = 0.134$, df = 16) differed significantly between these species, but *A. geoffroyi* had higher values in both parameters (Fig. 3). However, when the values were controlled for body mass$^{0.25}$, *A. geoffroyi* had a significantly longer MRT and MGT than *A. jamaicensis* (Fig. 3; MRT: $t = 2.93$, $P = 0.009$, df = 16; MGT: $t = 2.49$, $P = 0.024$, df = 16).

In *A. geoffroyi*, the percentage of empty grains was positively correlated with MRT ($r = 0.86$, $F = 24.54$, $P < 0.0001$, df = 1, 9), and *A. jamaicensis* had a trend toward more empty grains in individuals with longer MRT ($r = 0.72$, $F = 5.45$, $P = 0.067$, df = 1, 5). In contrast, MGT and the percentage of empty grains were not correlated significantly in either of the two species (*A. geoffroyi*, $r = 0.089$, $F = 0.08$, $P = 0.789$, df = 1, 9; *A. jamaicensis*, $r = 0.62$, $F = 3.14$, $P = 0.136$, df = 1, 5). As in *P. ellipticum*, the percentage of empty grains increased asymptotically with time in the gut (Fig. 4).

Columnar cactus pollen digestive processing.—The Percentage of empty cactus pollen grains differed significantly among the four species of bats (Fig. 2; one-way ANOVA, $F = 11.64$, $P = 0.0003$, df = 3, 16), but only between *L. curasoae* and both *A. jamaicensis* and *S. lilium*, and between *A. geoffroyi* and *S. lilium* (Tukey's hsd test, $P < 0.05$). *L. curasoae* had the highest and *S. lilium* the lowest percentage of empty grains among the four species of bats. There were no significant differences in MRT and MGT among the four species of bats (Fig. 3; one-way ANOVA, MRT: $F = 0.25$, $P = 0.8586$ df = 3, 16; MGT: $F = 2.01$, $P = 0.152$, df = 3, 16). MRT and MGT were not signifi-

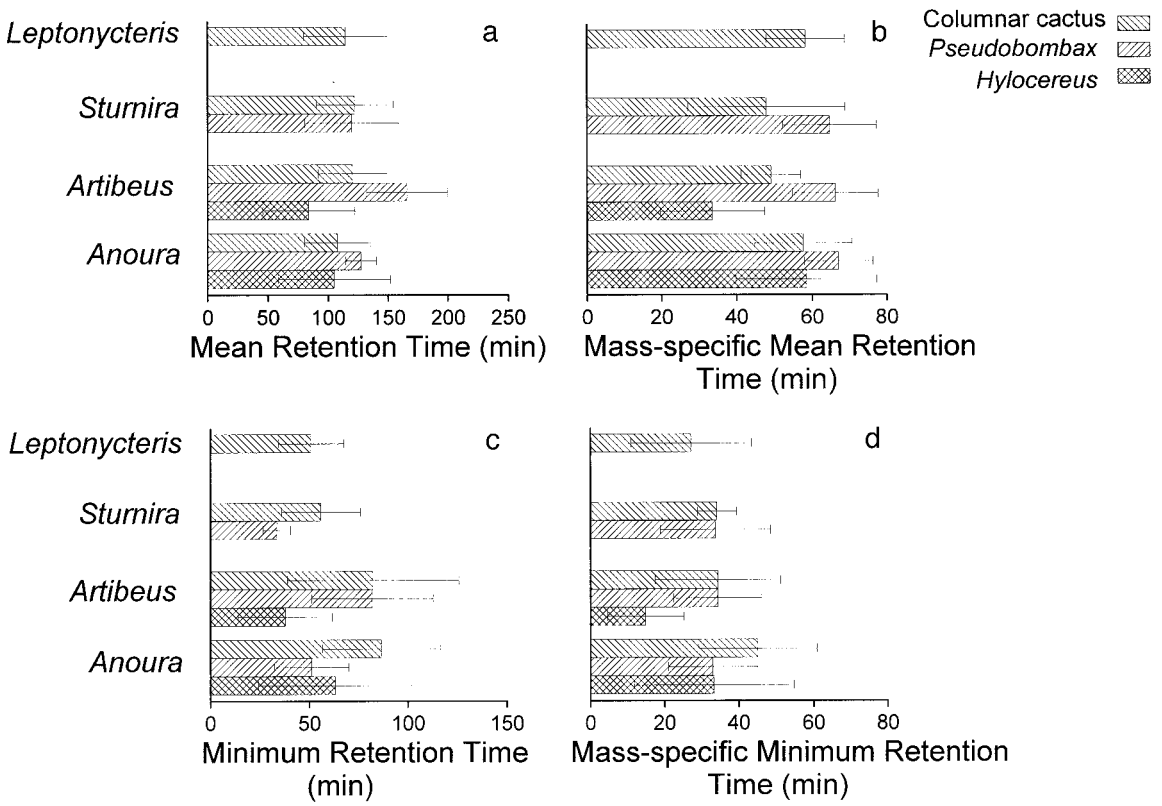

FIG. 3. Time distribution of pollen digestive processing for each species of bat and pollen type: (a) mean retention time; (b) mean retention time/body mass$^{0.25}$; (c) minimum gut transit time; and (d) minimum gut transit time/body mass$^{0.25}$. Error bars correspond to ± 1 SD.

cantly different among species even when the values were controlled for body mass$^{0.25}$ (Fig. 3; one-way ANOVA, MRT: $F = 0.78$, $P = 0.521$, df = 3, 16; MGT: $F = 1.36$, $P = 0.290$, df = 3, 16). Except in *L. curasoae* for MRT, the percentage of empty grains was correlated with neither MRT nor MGT in any of the species (MRT: *L. curasoae*, $r = 0.977$, $P = 0.0216$; *A. geoffroyi*, $r = 0.599$, *A. jamaicensis*, $r = 0.73$, *S. lilium*, $r = 0.366$; MGT: *L. curasoae*, $r = 0.022$, *A. geoffroyi*, $r = 0.186$, *A. jamaicensis*, $r = 0.529$, *S. lilium*, $r = 0.05$; all $P > 0.16$, df = 1, 4). The lack of statistical significance in some of these correlations is probably the result of small sample sizes. Individuals of *L. curasoae* with a longer MRT extracted a larger proportion of pollen contents. There was no apparent trend toward an increase in percentage of empty grains with time in the gut in any of the species; percentage of empty grains reached its peak in the first 50–75 min (Fig. 5).

Comparative processing efficiency of the three species of pollen by A. jamaicensis *and* A. geoffroyi.—In general, the three types of pollen were processed with the same efficiency and at the same rates, with some exceptions. Although *A. jamaicensis* emptied the same percentage of grains in the three pollen species (one-way ANOVA, $F = 3.49$, $P = 0.057$, df = 2, 15), *A. geoffroyi* emptied fewer grains of *H. undatus* pollen than of the other two pollen types (one-way ANOVA, $F = 10.13$, $P < 0.0001$, df = 2, 20; pairwise T' test for equal variances and unequal sample sizes: *H. undatus*–*P. ellipticum*, $P < 0.0001$; *H. undatus*–columnar cactus, $P = 0.010$; *P. ellipticum*–columnar cactus, $P > 0.100$). There were no significant differences among the MRT of the three types of pollen in *A. geoffroyi* (one-way ANOVA, $F = 0.74$, $P = 0.489$, df = 2, 20) and *A. jamaicensis* (one-way ANOVA, $F = 3$, $P = 0.08$, df = 2, 15). However, *A. jamaicensis* had significantly longer MRT for *H. undatus* than for *P. ellipticum* grains when the values were controlled for body mass$^{0.25}$ (one-way ANOVA, $F = 10.45$, $P = 0.001$, df = 2, 15; T' test: *H. undatus*–*P. ellipticum*, $P < 0.010$; *H. undatus*–columnar cactus, $P < 0.100$; *P. ellipticum*–columnar cactus; $P > 0.100$). Mean retention times controlled for body mass$^{0.25}$ were not significantly different among the three types of pollen in *A. geoffroyi* (one-way ANOVA, $F = 0.68$, $P = 0.517$, df = 2, 19). Mean gut transit times were not significantly different among pollen types in *A. geoffroyi* even after controlling for body mass (one-way ANOVA; MGT: $F = 1.71$, $P = 0.208$, df = 2, 19; MGT/mass$^{0.25}$: $F = 0.88$, $P = 0.423$, df = 2, 19), but

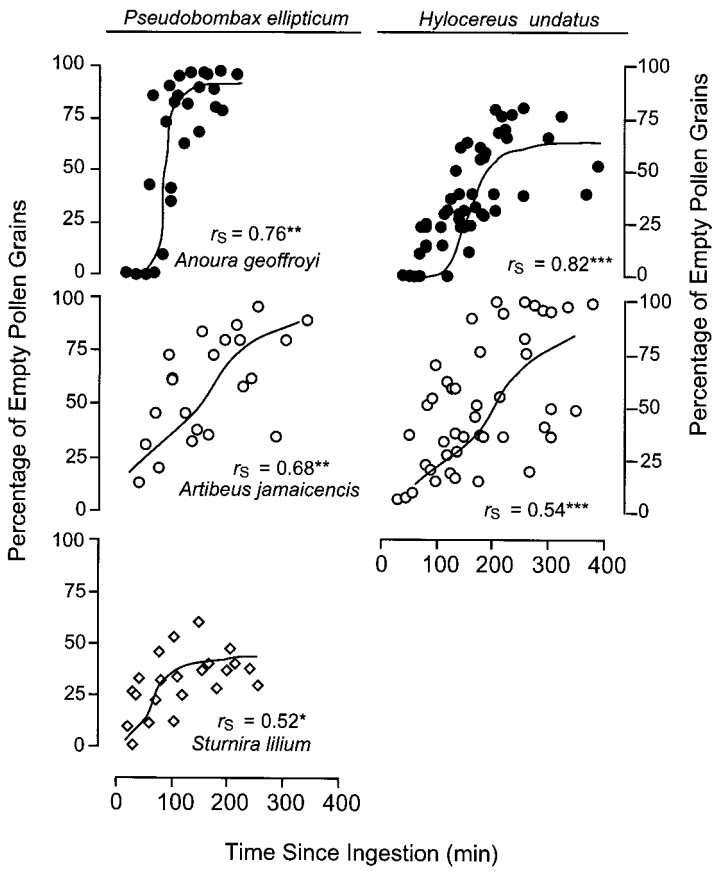

FIG. 4. Relationship between the percentage of empty pollen grains of *Pseudobombax ellipticum* and *Hylocereus undatus* in feces and time spent in the digestive tract. Curves were fitted with the SPLINE fitting procedure (SAS 1995). Points represent individual measurements and were used to calculate Spearman's coefficients of rank correlation. Significance levels are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

H. undatus grains had significantly shorter MGTs than those *of P. ellipticum* and the columnar cactus in *A. jamaicensis* (one-way ANOVA; MGT: $F = 4.25$, $P = 0.034$, df = 2, 15; T' test: *H. undatus–P. ellipticum* $P < 0.05$; *H. undatus*–columnar cactus, $P < 0.05$; *P. ellipticum*–columnar cactus, $P > 0.30$; MGT/mass$^{0.25}$: *H. undatus–P. ellipticum*, $P < 0.05$; *H. undatus*–columnar cactus, $P < 0.05$; *P. ellipticum*–columnar cactus, $P > 0.20$).

Excretion patterns.—In general, individual *F* curves resembled qualitatively the excretion curves expected for a CSTR-PFR reactor (Fig. 6). The slope of the plot of the log-transformed number of grains excreted (k, where the slope has been multiplied by -1 to make k a positive number) tended to be steepest in *A. geoffroyi*, followed by *S. lilium, L. curasoae,* and *A. jamaicensis* (Fig. 7; Table 1), and the average amount of time pollen grains spent in the stomach ($1/k$) was 15–75% of MGT, depending upon the species and pollen type (Table 1). In most cases, the sum of MGT and retention time in the stomach was only 68–99% of the MRT (Table 1). The mean amount of longitudinal mixing varied with type of pollen and species of bat; *A. jamaicensis* had intermediate to nearly large amounts of mixing, *S. lilium* and *L. curasoae* had intermediate amounts of mixing, and *A. geoffroyi* had small to intermediate amounts of mixing (Table 2).

DISCUSSION

Excretion patterns and chemical reactor models

In general, the grastrointestinal tracts of the bats we studied seemed to function as CSTR-PFRs with some longitudinal mixing in the PFR. In an ideal CSTR-PFR, MRT equals stomach mean retention time ($1/k$) plus MGT. This condition is almost satisfied in the digestive processing of pollen of *Hylocereus undatus* by *Anoura geoffroyi* and of columnar cactus pollen by all of the bats that we studied: MGT and $1/k$ together account for 85–99% of the MRT. There were small to nearly large amounts of longitudinal mixing in the digestive processing of these types of pollen. In the case of *Pseudobombax ellipticum* pollen processing, MGT and $1/k$ represented only 68–74% of the MRT. The amount of longitudinal mixing in the processing of this type of pollen was intermediate. Pollen grains were apparently retained in the stomach for slightly more than one-third of the MRT and only one-fifth, in the case of *A. geoffroyi.* Rainbow Lorikeets process nectar in a similar fashion: the food remains in the stomach for about one-fourth of the MRT and then it flows along the intestine,

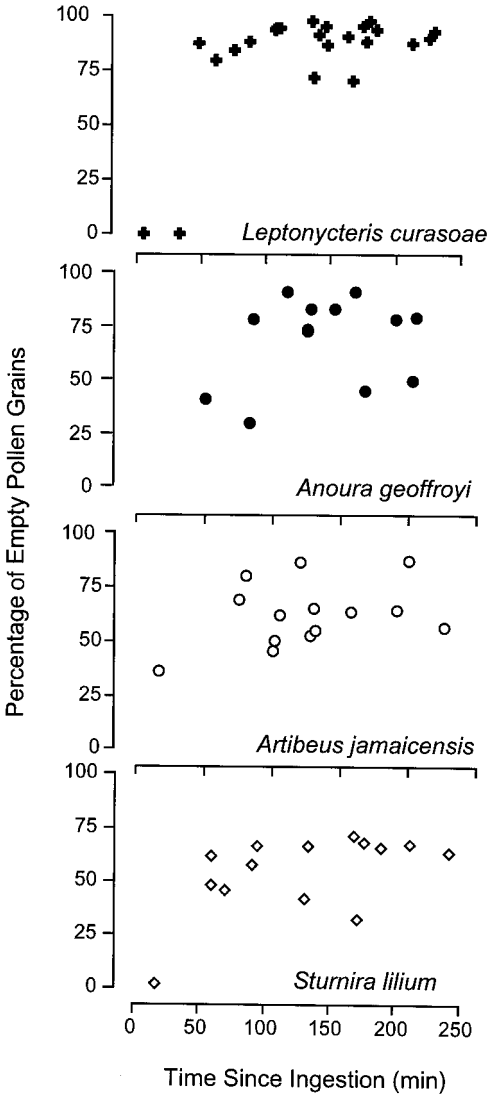

FIG. 5. Relationship between the percentage of empty pollen grains of columnar cactus in feces and time spent in the digestive tract. Points represent individual measurements. Note the sharp increase in the percentage of empty grains as a function of time.

probably with some longitudinal mixing (Karasov and Cork 1996). Longitudinal mixing also occurs in the intestine of frugivorous birds (Levey and Duke 1992).

The only exception to this pattern of a gastrointestinal tract that functions as a CSTR-PFR, with short mean retention times in the stomach and longitudinal mixing in the intestine, was *Artibeus jamaicensis* when given *H. undatus* pollen. According to the PFR-CSTR model, this species had a very large mean retention time in the stomach (60 min), which accounted for three-quarters of the MRT; we consider this rather unlikely, given that most digestion of pollen occurs in the intestine (Law 1992*a*). We suggest that the gastrointestinal tract, in this case, functioned as a single PFR with a large amount of longitudinal mixing. PFRs with large amounts of longitudinal mixing produce output distribution curves similar to the curves of CSTRs (Martínez del Rio et al. 1994). In this case, the vessel dispersion number was slightly lower than the value characteristic of gastrointestinal tracts with high amounts of longitudinal mixing.

It is important to mention that there were a few cases in which the first excretion contained nearly 100% of the total number of grains excreted, and the bat gastrointestinal tract seemed to function as a simple PFR. In Queensland blossom bats (Pteropodidae: *Syconycteris australis*), two different kinds of pollen were excreted in different fashions: *Callistemon* grains were excreted almost completely in the first excretion, whereas the first excretion of *Banksia* grains contained only 60–70% of the total number of grains ingested (Law 1992*a*).

Pollen extraction and processing time

Extraction of pollen contents was higher in bats that are specialized flower visitors (*L. curasoae* and *A. geoffroyi*) than in seasonal flower visitors (*A. jamaicensis* and *Sturnira lilium*), even though they processed pollen grains at approximately the same rates. *L. curasoae* and *A. geoffroyi* emptied 46–90% of the pollen grains with a MRT that ranged from 105 to 127 min and a MGT ranging from 50 to 88 min, whereas *A. jamaicensis* and *S. lilium* extracted the contents of 32–68% of the pollen grains with a MRT of 101–166 min and a MGT of 32–82 min. These results suggest that phyllostomid nectarivores have a digestive system that allows them to feed more efficiently on pollen grains than do their frugivorous relatives. In contrast, Wooller et al. (1988) found no differences in pollen extraction when they compared species of birds that regularly ingest pollen with species that do not. They concluded that pollen eating did not require a specialized digestive system, but their study failed to use a standard methodology for the comparison.

Nectarivorous bats in this study had higher extraction levels of pollen contents than did pteropodid nectarivores (53–57%; Law 1992*a*), rats (52–59%; Bell et al. 1983), sugar gliders (53–59.3%; Goldingay et al. 1987, Goldingay 1990), pygmy possums (63–78%; Turner 1984), and honeyeaters, finches, Budgerigars, lorikeets, Cockatiels, and hummingbirds (<7–48%; Brice et al. 1989, Wooller et al. 1988). In fact, the pollen extraction of the highly derived nectarivorous species, *L. curasoae*, is close to the extraction of honey possums (95–100%; Turner 1984, Richardson et al. 1986). This level of pollen extraction is achieved with a much shorter gut retention time (peak transit time is 6 h in honey possums with a body mass of 14 g; Richardson et al. 1986). The other reported vertebrate nectarivores with a similar degree of pollen extraction are two species of Darwin's finches (>90%; Grant 1996).

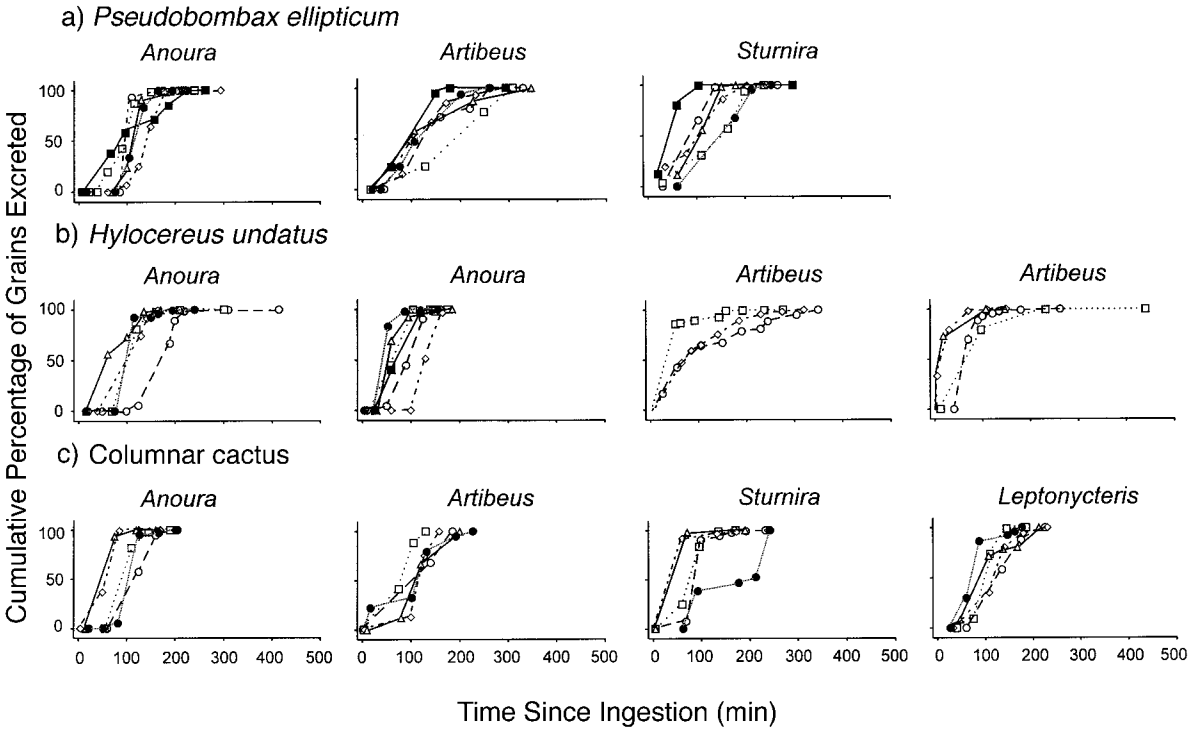

FIG. 6. Cumulative excretion curves (*F* curves) of ingested pulses of three different pollen types in four species of bats; each curve represents an individual bat. The three types of pollen were from (a) *Pseudobombax ellipticum,* (b) *Hylocereus undatus,* and (c) columnar cactus.

This study, however, provided no estimates of gut transit time and, thus, limits possible comparisons.

In general, the digestive system of individuals with longer gut retention times (MRT or MGT) did not empty more pollen grains. Exceptions to this pattern were *A. geoffroyi* and *L. curasoae*; these species exhibited a positive relationship between MRT and the percentage of empty grains of *H. undatus* and columnar cactus pollen, respectively. In addition to this, *A. jamaicensis* had a trend ($P = 0.067$) toward more empty grains of *H. undatus* pollen in individuals with longer MRT. When individual measurements were examined, however, the percentage of empty grains increased asymptotically with time spent in the gut for both *H. undatus* and *P. ellipticum* pollen, which suggests the existence of a trade-off between assimilation and food processing rate (Martínez del Rio et al. 1989, Karasov and Levey 1990). If food is processed too quickly in the gut, its assimilation seems to become compromised. With pollen from columnar cactus, plotting of individual measurements showed no apparent increase in the percentage of empty grains with time in the gut, but a peak in the first 50–75 min, followed by a plateau. Lack of a significant relationship between gut processing time and pollen extraction has been demonstrated previously in Queensland blossom bats (Law 1992*a*). In contrast, time spent in the gut increases extraction of pollen contents in honey possums (Richardson et al. 1986). In view of the apparent lack of generality in the correlation between extraction of pollen contents and residence time in the gut, and without information on the mechanisms by which bats, and other vertebrates, extract pollen contents, our evidence for a trade-off between pollen digestion and gut retention time must be considered tentative.

Most extraction of pollen contents by bats and other vertebrates occurs in the intestine (Richardson et al. 1986, Law 1992*a*), probably by enzymatic action on the pollen wall, followed by osmotic shock (Peng et al. 1986). The minimal digestion of pollen in the stomach (Law 1992*a*) offers no support to the role of acid-secreting cells in the stomach of bats as a mechanism for the extraction of pollen contents (Howell 1974*a*). Given that the bats that we studied processed the grains at about the same rate, and that they had similar stomach retention times, differences in their extraction efficiency may be accounted for by differences in the activity level of the intestinal enzymes responsible for pollen wall degradation.

Contrary to what Law (1992*a*) found in Queensland blossom bats, contents of different pollen types were extracted with a different efficiency by two species of bats. Extraction of contents by *A. geoffroyi* was higher with *H. undatus* pollen than with *P. ellipticum* and columnar cactus pollen, although the three types of pollen were processed at the same rate. *A. jamaicensis*

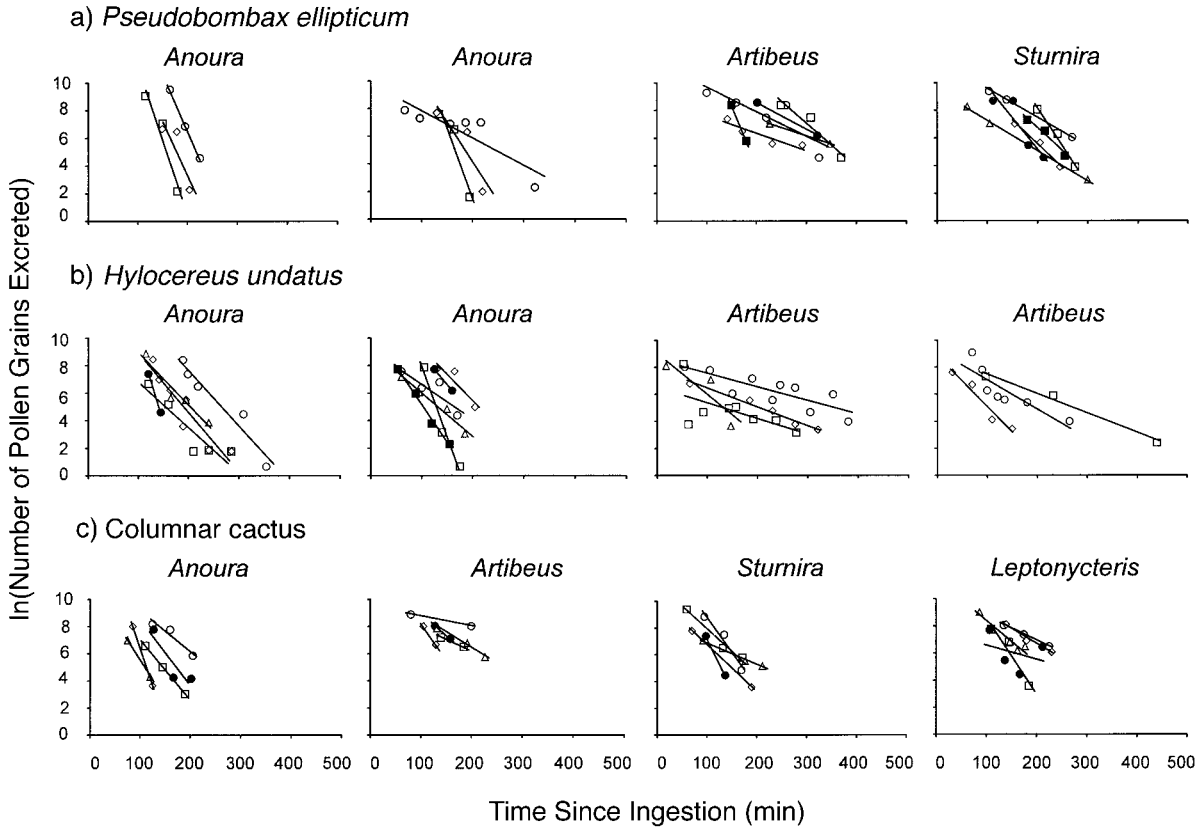

FIG. 7. Linear regression of the terminal portion of the output distribution curves of ingested pulses of pollen. Different symbols and fitted lines represent individual bats. Distribution curves were the ln-transformed number of pollen grains excreted since ingestion, and the regression began at the highest number of grains excreted and continued to the right of this peak. The slopes of each regression were used to estimate stomach retention times for (a) *Pseudobombax ellipticum*, (b) *Hylocereus undatus*, and (c) columnar cactus.

had a trend ($P = 0.057$) toward a decrease in digestion of *H. undatus* pollen, explained partly by significantly shorter MRT and MGT values compared to the other two species of pollen. Although the flowers of *H. undatus* possess moth- rather than bat-pollination characteristics (white, large, tubular, nocturnal flowers with an intensely sweet odor; Rowley 1980), their pollen may be a regular part of the diet of *L. curasoae* when chiropterophillous plants are not blooming (Valiente et al. 1996). It is noteworthy that the pollen of what ap-

TABLE 1. Residence time in minutes (mean ± 1 SD) of each pollen type in the stomach of each species of bat. Terms are as follows: k is the slope of the linear regression of the output distribution curves (Fig. 7), multiplied by −1 to make k a positive number. The inverse of k represents mean retention time in the stomach. MRT and MGT are mean gut retention times and minimum gut transit times, respectively.

Pollen types and bats	k	k^{-1}	k^{-1}/MRT	k^{-1} + MGT/MRT
Pseudobombax				
Artibeus jamaicensis	0.032 ± 0.01	37.49 ± 22.2	0.282 ± 0.12	0.747 ± 0.19
Anoura geoffroyi	0.054 ± 0.01	18.94 ± 8.5	0.156 ± 0.05	0.715 ± 0.16
Sturnira lilium	0.035 ± 0.01	30.67 ± 10.6	0.285 ± 0.11	0.688 ± 0.28
Hylocereus				
Artibeus jamaicensis	0.020 ± 0.01	60.92 ± 26.9	0.752 ± 0.21	1.398 ± 0.32
Anoura geoffroyi	0.048 ± 0.02	24.72 ± 9.44	0.242 ± 0.13	0.944 ± 0.19
Columnar cactus				
Artibeus jamaicensis	0.030 ± 0.01	41.16 ± 18	0.316 ± 0.11	0.906 ± 0.22
Anoura geoffroyi	0.051 ± 0.01	21.24 ± 6.9	0.187 ± 0.04	0.996 ± 0.28
Sturnira lilium	0.032 ± 0.01	36.12 ± 16.4	0.403 ± 0.03	0.941 ± 0.08
Leptonycteris curasoae	0.029 ± 0.01	39.52 ± 14.9	0.304 ± 0.08	0.854 ± 0.13

TABLE 2. Degree of longitudinal mixing in the gastrointestinal tract. Values are mean ± 1 SD of the vessel dispersion number ($D/\mu L$). Existence of no longitudinal mixing is indicated by a $D/\mu L$ value equal to zero, and there is evidence of mixed flow as the value approaches infinity (Levenspiel 1972).

Bat species	Columnar cactus	*P. ellipticum*	*H. undatus*
A. jamaicensis	0.034 ± 0.03	0.062 ± 0.02	0.158 ± 0.13
A. geoffroyi	0.009 ± 0.00	0.031 ± 0.02	0.025 ± 0.02
S. lilium	0.046 ± 0.02	0.044 ± 0.02	
L. curasoae	0.031 ± 0.01		

pears to be a moth-pollinated species was assimilated less efficiently by bats.

Because certain Old and New World nectarivorous bats and sugar gliders can maintain positive nitrogen balances on a pollen–nectar diet (Howell 1974*a*, Law 1992*b* Smith and Green 1987), pollen is probably an important source of nitrogen in the diet of some flower-visiting animals. Similarly, pollen consumption in Darwin's finches has significant implications for their successful breeding (Grant 1996). Efficient digestion of pollen may thus be critical to some animals that rely on this resource. In this study, the efficiency of extraction of pollen contents was positively related to the degree of flower specialization of the bats. All of the species were capable of extracting pollen contents, but the most specialized nectarivorous and pollenivorous species were the most efficient: their digestive system emptied a higher percentage of pollen grains than the digestive system of the two frugivorous species, even though food was processed at the same rate.

We found consistent differences in pollen digestive processing between species of bats that regularly feed on pollen and species that are seasonal pollen consumers. In general, regular pollinivores digested a higher fraction of pollen grains than did seasonal pollinivores. However, we could not explain these differences by the rate at which pollen was processed, or by excretion patterns according to chemical reactor models. Except in a few cases, the percentage of empty grains was not related to processing time when mean values were considered for each species of bat and plant. Nevertheless, at least in some cases, pollen extraction increased asymptotically with processing time when individual measurements were analyzed. The slope of this pollen extraction vs. retention time relationship may provide a good comparative estimator to assess the ability of different pollinivores to extract pollen contents. Such a comparison, however, awaits a larger species sample. We believe that future comparative studies on pollinivory must address two issues: (1) what are the biochemical processes involved in pollen wall degradation, and (2) what are the nutritional benefits that flower-visiting animals derive from pollen? Understanding the digestive and metabolic traits exhibited by pollen-feeding animals may deepen our understanding of the factors that mold the relationships between pollinators and plants.

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