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POLLEN AGGREGATION IN RELATION TO POLLINATION VECTOR

David Timerman,[1,*] David F. Greene,† Josef D. Ackerman,‡ Peter G. Kevan,§ and Erika Nardone§

*Department of Biology, Concordia University, Montreal, Quebec, Canada; †Department of Geography, Planning and Environment, Concordia University, Montreal, Quebec, Canada; ‡Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada; and §School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

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Premise of research. Angiosperms possess pollen dispersal units (PDUs) of varying size, from monads (single grains) to aggregates containing thousands of grains. It has been suggested that the degree of aggregation is related to the dispersal agent (in particular, animals vs. wind), but aggregation has rarely been measured, and its correlation with pollination vectors has been examined for only a few species.

Methodology. Assuming a lognormal distribution, the expected distribution for a random disaggregation process, we tested the hypothesis that the distribution of PDU sizes depends on the pollination vector, using 32 anemophilous and zoophilous species. We also examined intraspecific variation in the lognormal parameter values, using 30 different individuals of the common weed *Plantago lanceolata*.

Pivotal results. The mean and standard deviation of the lognormal distribution of PDUs and a third parameter, the proportion of solitary monads, all separated the species by pollination vector, with the mean slightly outperforming the other two metrics. The majority of species (75%) had a PDU distribution that was not significantly different from a lognormal expectation, as did most *P. lanceolata* individuals (57%). The proximate reason for the lack of fit of species with the lognormal was the overrepresentation of monads for many of the anemophilous species; a monad (1.0) represents the lower bound for a disaggregation process, and the lower limit for the lognormal is 0. Interestingly, the two *Plantago* species examined, thought by some authors to be ambophilous (using both wind and animals for dispersal), had lognormal parameter values intermediate between those for the animal- and wind-pollinated species.

Conclusions. Our results indicate that aggregate size is a promising quantitative measure for distinguishing pollination vectors. Future work should focus on other factors governing PDU size, such as relative humidity and time since anther dehiscence, use a common methodology for generating disaggregation, and focus on putative ambophiles to ascertain whether they do indeed have a degree of aggregation intermediate between those of species whose pollen is dispersed solely by wind and species whose pollen is dispersed by animals.

Keywords: pollen aggregation, pollen clumping, pollen dispersal, anemophily, zoophily.

Introduction

Pollen characteristics such as size and exine ornamentation are important in plant reproduction (Muller 1979; Harder 1998; Edlund et al. 2004). Of particular significance for plant mating is the number of pollen grains that are dispersed by pollination vectors as cohesive "pollen dispersal units" (PDUs); these may range from single grains (monads) to units of thousands of grains (Willson 1979). The distribution of PDU sizes can have important genetic and ecological consequences; for example, the size of units arriving on the stigmas of multiovulate flowers may result in varying levels of shared paternity among progeny (Ritland 1989; Morgan and Barrett 1990). PDU size may also restrict the total number of pollen recipients and fertilization opportunities for individuals when PDU size exceeds the ovule number per pistil, as not all pollen grains will sire seeds (Pacini 2000). Despite the importance of these and other effects (see Harder and Johnson 2008), little is known empirically about the variation within and among species in PDU size distribution. Further, it has often been argued that the size distribution is adaptive for a particular pollination mode (e.g., wind or animal), but this hypothesis has not been examined rigorously.

The shape of the probability distribution of PDU sizes for angiosperms is related to the aggregation mechanisms, and these can involve (1) adhesion via pollenkitt, (2) adhesion via filaments from the exine or anther wall, and (3) lack of separation of cells in microsporogenesis (Pacini 2000). In some cases only a single size class is produced, whereas in other instances a range of PDU size classes are evident. For example, mechanism 3 would result in only the polyad PDU size class.

[1] Author for correspondence; current address: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada; e-mail: david.timerman@mail.utoronto.ca.

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In contrast, species with mechanisms 1 or 2 are expected to have a much more varied distribution of PDU sizes, since these mechanisms do not act on pollen grains equally throughout the anther. Further, once PDUs are entrained by the wind or are in contact with a pollinator, they begin to disaggregate (Lisci et al. 1996; Harder and Johnson 2008; Hesse 2010), and this susceptibility to disaggregation may well be controlled via the mechanism by which units cohere. For example, if an anemophilous PDU is held together mainly by moisture, then small-scale eddies in a low-humidity air column could lead to rapid disaggregation with distance (Martin et al. 2009). We note that, to the best of our knowledge, all studies of the PDU size distribution deal with postrelease analysis. There may be differing aggregating mechanisms that lead to species-specific, prerelease distributions.

Zoophilous species tend to disperse aggregated pollen, whereas anemophilous species are more likely to disperse solitary monads (Faegri and van der Pijl 1966; Ackerman 2000; Pacini 2000; Harder and Johnson 2008), although exceptions have been noted (Buchmann and Hurley 1978; Stelleman 1984*b*; Lisci et al. 1996; Martin et al. 2009; Michalski and Durka 2010). Presumably, ambophilous (wind- and animal-pollinated) species would have an intermediate amount of clumping. There are a number of explanations as to why anemophilous plants should have less pollen aggregation, the most common argument being that aggregation may be disadvantageous aerodynamically for anemophilous plants. More specifically, larger PDUs have higher settling velocities than solitary grains, resulting in, on average, shorter dispersal distances and thus reduced gene flow (Niklas 1985; Tonsor 1985*a*, 1985*b*; Di-Giovanni et al. 1995; Jackson and Lyford 1999; Martin et al. 2009). It could also be that aggregated pollen is disadvantageous aerodynamically because a greater force may be required to remove aggregates from anthers during anthesis (Niklas 1985). Conversely, a larger clump would be more susceptible to the force of drag (proportional to frontal area), would project farther into the boundary layer, and thus would be more likely to abscise. Interestingly, only two empirical studies of release in relation to PDU size have been undertaken, and both found that pollen release was enhanced by aggregation in the anemophilous *Ambrosia* (Martin et al. 2009; Sabban et al. 2012). In contrast to anemophily, pollen aggregation is advantageous in biotic pollination (Pacini 2000; Harder and Johnson 2008), as it can, for example, improve siring success by increasing the adhesion of pollen to floral visitors (via adhesive substances), reducing pollen loss during grooming (via threaded pollen or pollinia), and delivering multiple pollen grains to stigmas (e.g., zoophilous ovaries are often multiovulate, whereas anemophilous ovaries are invariably uniovular). Although these functional arguments reasonably account for why there should be differences between pollination modes, there has been to date only a single, small-sample ($n = 6$ species) study examining the relationship between the PDU size distribution and pollination system (Hall and Walter 2011).

One challenge in measuring PDU size distributions for the purpose of interspecific comparison is that disaggregation begins within the anther following dehiscence and subsequently continues during the release and dispersal phases. Thus, differences in the stage when PDUs are sampled may confound comparisons. We propose that by applying the same large mechanical impulse to samples and then having a constant fall distance ("dispersal"), we can at least bring some uniformity to the stage at which we sample for PDU sizes. If aggregation is stronger among zoophilous plants, then there should be less disaggregation than among anemophilous plants, where aggregation may be maladaptive for pollen dispersal. More fundamentally, a standardized approach is useful because the parameters of the resulting distribution of PDU sizes can be regarded as a proxy measure of the strength of the adhesive forces, such as pollenkitt, resisting disaggregation.

To the best of our knowledge, no one has proposed a probability distribution for PDUs. We anticipate that all sample size distributions will follow a lognormal distribution, since the subdivision of PDUs should be a multiplicative process, as with the breaking up of saltating sand grains on a riverbed (Visher 1969). This will lead to a right-skewed distribution that tends to a lognormal distribution with increasing disaggregation events (King 1981). However, there are three situations where the probability of PDU sizes would be right skewed but not necessarily lognormal: (1) disaggregation is strongly nonrandom with respect to PDU size, (2) there has been too little time for the disaggregation process to operate, and (3) too much time has elapsed so that monads, the lower limit for the disaggregation process, become very numerous. The last is a problem, as the lognormal has 0 as a lower bound.

In this article, we examine the PDU size distribution for a large number of anemophilous and zoophilous species to determine whether the degree of pollen aggregation segregates by pollination vector. Specifically, we predict that values for the two parameters of the lognormal PDU size distribution, the mean and standard deviation of the logarithms, will be much smaller for anemophilous plants. We also examine the PDU size distribution for an anemophilous species, *Plantago lanceolata*, by comparing anthers from many individuals. *Plantago lanceolata* was chosen for analysis because there is some evidence that populations vary in their pollination system from anemophilous to ambophilous (Stelleman 1984*b*). We predict that this species should have intermediate lognormal parameter values between those of anemophilous and zoophilous species. Finally, we examine whether the lognormal adequately describes the distribution of PDU sizes.

Material and Methods

Intraspecific Analysis of Plantago lanceolata

Plants of *P. lanceolata* were grown at Concordia University in Montreal, Quebec, in 10 × 10-cm pots from seeds obtained from Horizon Herbs (Williams, OR). Flowering was induced over 3 months in a growth chamber with 16-h daylight intervals at 25°C and 8-h dark intervals at 21°C. When flowering was observed in April 2012, the plants were transferred to a laboratory, where they were kept beneath a timer-controlled fluorescent lamp programmed at the same lighting schedule as noted above. Relative humidity in both environments varied between 50% and 60%. Plants were experimented on in the laboratory within 3 days of being transferred.

We applied standardized forces to pollen samples to initiate the disaggregation process. These methods were used to pro-

duce "sprinkling images" of pollen dispersed on microscope slides, and then the resulting distributions of PDU sizes were measured. The first of these methods vibrated pollen from 30 anthers from 30 individuals of *P. lanceolata*. Shortly after dehiscence, typically between 9:00 and 10:00 a.m. (local time), flowers were removed from the inflorescence by plucking them at the receptacle base with fine-tipped forceps. We were careful to not dislodge pollen from the anthers. The bases of these flowers were then embedded in an adhesive (Lepage Fun-Tak, Mississauga, ON, Canada). Each was then mounted horizontally onto a vertically oriented SmartShaker Pro K2004E01 electrodynamic shaker (Cincinnati). Three of the four stamens and the carpel were removed from the flower with forceps. A microscope slide coated with silicon grease was then placed 1 cm from the anther at a 45° angle. A BK Precision 4003A signal generator (Yorba Linda, CA) was used to vibrate the shaker sinusoidally through increasing frequencies at low amplitude until the stamen began to resonate. The excitation was then amplified until the resonating stamen ejected pollen onto the microscope slide. The falling grains were captured on the microscope slide and examined under light microscopy to generate a frequency distribution of PDU size (grains per PDU). Frequencies of pollen grains per PDU were grouped into 11 integer-bounded base-2 logarithmic bins.

Interspecific Analysis

We measured the disaggregation of pollen sampled from flowers with intact anthers from 23 species (table 1) that were in bloom between November 27 and December 2, 2010, around the Pró-Mata Research and Nature Conservation Center in Rio Grande do Sul, Brazil. All plants were identified to species level, with the exception of three Poaceae, which were classified as separate species on the basis of morphological and phenological differences. The specimens were kept in water on a lab bench beneath an incandescent lamp until dehiscence could be observed with a hand lens (usually within 2 h). Relative humidity during this period ranged from between 50% and 90%. The designations of pollination vectors were based to some degree on observations at the field station but were mainly inferred from the macroscopic floral traits of specimens (i.e., pollination syndromes). Although we observed the frequent visitation of syrphid flies on the flowers of *Plantago major*, it was included with the anemophilous plants since, to our knowledge, no study has shown ambophily in this species.

We followed the technique of Stelleman (1984*b*) and Ackerman and Kevan (2005) to produce sprinkling images of pollen dispersed on microscope slides. Briefly, pollen from the newly dehisced anther was shaken through a 1.91-cm-diameter hole bored lengthwise through a 30-cm-long aluminium cylinder in which an acrylic disk was placed at half the cylinder length (see Ackerman and Kevan 2005). The cylinder was then inverted by 180° and lowered to 1 mm above a microscope slide coated with a thin layer of silicon grease. A 1.40-cm-diameter steel ball bearing was dropped through the top of the cylinder, where it collided with the acrylic disk, and the shock dislodged the pollen on its underside. The falling grains were trapped on the microscope slide and examined under light microscopy to generate a frequency distribution of PDU size (grains per PDU) as described above.

Table 1

Plant Species Organized by Pollination Vector

Pollination vector, species	PDUs	$\overline{X}$	SD	g_1	DI	*D*
Anemophilous:						
Araucaria cunninghamii[a,b]	113	.59	.85	10.39	.58	.232
Pinus tecunumanii[a,b]	68	.69	1.27	5.43	.52	.283
Casuarina cunninghamiana[a]	296	.62	1.19	5.93	.54	.282*
Gunnera manicata	50	.5	.00		1.00	
Plantago major	90	.93	1.15	4.13	.17	.263*
Poaceae species 1	822	.66	.39	2.29	.73	.040
Poaceae species 2	102	.65	.36	1.98	.95	.014
Poaceae species 3	119	.53	.16	6.03	.74	.024
Zea mays[a]	92	.48	.82	10.74	.65	.229
Mean		.63	.69	5.87	.65	
Plantago lanceolata	172	1.4	.97	1.51	.21	
Zoophilous:						
Cycas revoluta[a,b]	75	1.51	2.46	.61	.08	.307
Lepidozamia peroffskyana[a,b]	246	.99	1.80	2.59	.25	.309*
Baccharis trimera	10	2.2	2.31	.69	.05	.298
Begonia cucullata	23	5.11	1.41	.22	.00	.053*
Buckinghamia celsissima[a]	59	1.31	1.98	1.75	.14	.253
Coccocypselum condalia	13	2.04	1.05	.11	.04	.053
Fuchsia regia	40	1.98	1.45	.76	.06	.193
Hymenocallis littoralis[a]	26	2.71	2.62	.56	.05	.178
Ilex paraguariensis	22	3.05	1.53	.86	.00	.610*
Jacobina carnea	15	2.97	2.72	.55	.02	.239
Lupinus magnistipulatus	77	2.62	1.35	1.24	.23	.102
Lupinus reitzii	71	1.19	1.44	2.59	.00	.271
Mutisia speciosa	72	1.85	1.90	1.48	.08	.190
Passiflora caerulea	66	2.89	2.56	.81	.01	.152
Roupala rhombifolia	136	1.31	1.15	1.45	.21	.179
Senecio brasiliensis	485	2.15	1.30	.60	.04	.048
Sinningia warmingii	93	5.89	1.94	.61	.00	.154*
Sisyrinchium palmifolium	43	6.2	1.79	−.06	.00	.068*
Sisyrinchium sellowianum	99	1.35	1.31	2.23	.19	.160
Solanum variabile	40	2.4	.93	−.38	.03	.158
Trixis lessingii	79	2.99	1.13	−.25	.01	.054
Vriesia platynema	88	3.49	1.58	2.64	.00	.225*
Mean		3.02	1.77		.06	

Note. Descriptive statistics are calculated for lognormal distributions (mean [$\overline{X}$], standard deviation [SD], and skewness [g_1] of the logarithms of pollen dispersal unit [PDU] size). The disaggregation index (DI) is a minimum estimate of the proportion of solitary grains (see "Material and Methods"). Species whose lognormal parameters and DI do not consistently predict the pollination vector (see "Results" for criteria) are underlined. Also shown is the Kolmogorov-Smirnov *D* statistic used to examine the goodness of fit to a lognormal distribution.

[a] From Hall and Walter (2011).

[b] Gymnosperms.

* Species distribution differed from lognormal ($P < 0.05$).

Statistical Analysis

To assess whether disaggregated pollen follow a lognormal distribution, the intra- and interspecific PDU size distributions were compared to a lognormal distribution with the Kolmogorov-Smirnov goodness-of-fit test. Data for 8 species from Hall and Walter (2011) were included in this analysis. Because their largest pollen PDU size bin was unbounded (>100 grains per PDU), we assumed that no PDU would exceed 2048

grains—an exceedingly rare upper bound for our observations in Rio Grande do Sul—in order to calculate the statistical moments of the lognormal distributions.

We used contingency tables to examine the hypothesis that the distribution of PDU sizes was independent of the pollination vector (anemophilous and zoophilous). To test this hypothesis, we grouped the data from Rio Grande do Sul into four PDU size classes (1, 2–3, 4–7, and >8 grains per PDU) to ensure that each cell had a minimum of five observations, satisfying the assumptions of the χ^2 test.

The "disaggregation index" (DI), a minimum estimate of the proportion of monads used by Hall and Walter (2011) to quantify aggregation, was calculated for each distribution by dividing the number of monads (f_m) by the sum of each of the 11 PDU size bins' frequency (f_i) multiplied by its lower bound (l_i; $\mathrm{DI} = f_m / \sum_{i=1}^{11} f_i l_i$). We examined whether there were similarities in the first two statistical moments (i.e., mean and standard deviation) of the lognormal distribution of PDU size and DI between anemophilous and zoophilous species by separate Mann-Whitney *U*-tests. Least squares linear regression was used to examine the relationship between the moments of the lognormal distribution of PDU size and DI for each pollination vector. Data from Hall and Walter (2011) were combined with those from the species studied here and also tested separately.

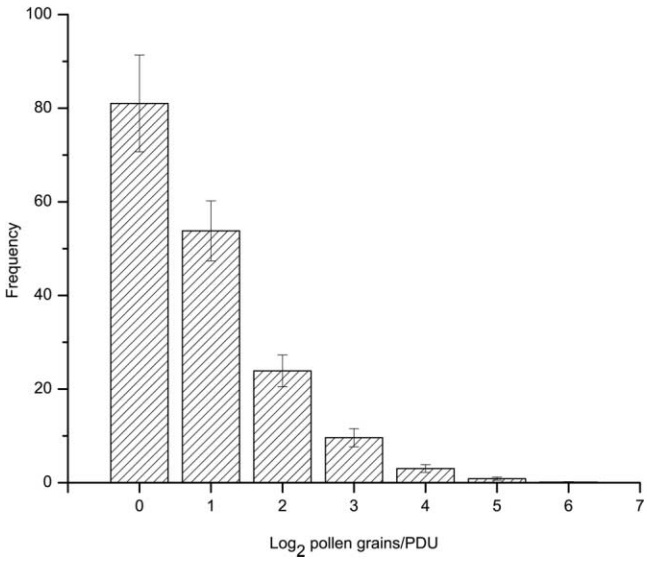

Fig. 1 Ensemble average (mean ± SE) of $\log_2$(pollen dispersal unit [PDU] size) for *Plantago lanceolata*. Bars are centered on bin lower bounds (bin width = 1).

Results

The pollen released from *Plantago lanceolata* was found to aggregate, with 172 ± 102 (average ± standard deviation) PDUs per anther (n = 30 individuals; a total of 5165 PDUs examined). The distributions of PDU sizes ranged from monads to 64 pollen grains in a markedly right-skewed fashion (skewness g_1 = 1.51; fig. 1). The mean PDU size was 2.64 ± 1.96 pollen grains per PDU (i.e., 1.4 ± 0.97 $\log_2$(pollen grains per PDU)) and the DI (proportion of monads) was 0.21 (table 1). Almost 60% of the PDU distributions fitted a lognormal distribution (Kolmogorov-Smirnov test; $P < 0.05$). The remaining flowers had PDU size distributions that were right skewed but not lognormal, primarily because of an overabundance of solitary monads.

Whereas pollen aggregation was found in anemophilous species (n = 5), it was more extensive in the zoophilous species (n = 18; i.e., a total of 23 Brazilian species), extending to PDU sizes of 1024 pollen grains per PDU, versus 512 among the anemophiles (fig. 2). The mean PDU was less for anemophilous species, including the data from Hall and Walter (2011), at 1.55 ± 1.61 pollen grains per PDU (n = 8; i.e., 0.63 ± 0.69 $\log_2$(pollen grains per PDU)), with a DI of 0.65. By contrast, the mean size for the zoophilous species was 8.11 ± 3.41 pollen grains per PDU (n = 22; i.e., 3.02 ± 1.77 $\log_2$(pollen grains per PDU)), with DI = 0.06 (table 1). The distributions were right skewed (table 1), especially among the anemophilous species. The majority of the species (75% of the anemophilous and 73% of the zoophilous species) had PDU size distributions that followed a lognormal distribution (χ^2; $P < 0.05$; table 1). The anemophilous species that did not fit the lognormal distribution had high proportions of monads.

As indicated above, the distribution of PDUs was dependent on the pollination vector ($X_3^2 = 132.51$, $P < 0.05$; n = 23), in that PDU sizes were larger among the zoophilous species (4.39 and 3.89 times as large for the mean and standard deviation, respectively; fig. 2; table 1). In addition, there were significant differences between the means and standard deviations, respectively, of the $\log_2$-transformed data for anemophilous and zoophilous species (Mann-Whitney U = 0.0; i.e., mean PDU sizes all tended to be larger in the zoophiles, P = 0.001; and U = 3.5, P = 0.002 for standard deviation). Similarly, the DI was 11.5 times as large for anemophilous as for zoophilous species and differed significantly between vectors (U = 3.0, P = 0.002). A significant linear relationship was found between DI and the mean and standard deviation of the logarithms of PDU size (r^2 = 0.40, n = 32, $P < 0.001$, for DI vs. mean; r^2 = 0.52, n = 32, $P < 0.001$, for DI vs. standard deviation; fig. 3). The overall trend was similar when the analysis was repeated for separate data sets (i.e., r^2 = 0.34, n = 23, P = 0.002 for DI vs. mean; r^2 = 0.52, n = 23, $P < 0.001$, for DI vs. standard deviation for the interspecific data set; and r^2 = 0.68, n = 8, P = 0.007 for DI vs. mean; r^2 = 0.95, n = 8, $P < 0.001$, for DI vs. standard deviation for the data from Hall and Walter 2011).

The use of the mean of the $\log_2$-transformed data permitted us to discriminate reliably (i.e., with no errors) among species by pollination vector (fig. 3*A*); a mean value of ~0.95 separated zoophilous from anemophilous species in both data sets. Similarly, a DI value of ~0.4 discriminated between the two pollination vectors, with one error (*Plantago major*). Conversely, the standard deviation of the $\log_2$-transformed data was less effective in discriminating between vectors, where a separation at ~0.85 resulted in misclassification of three anemophilous species (fig. 3*B*). Interestingly, the means of all 30 flowers, standard deviation, and DI for the anemophilous *P. lanceolata* specimens placed it among the zoophilous species.

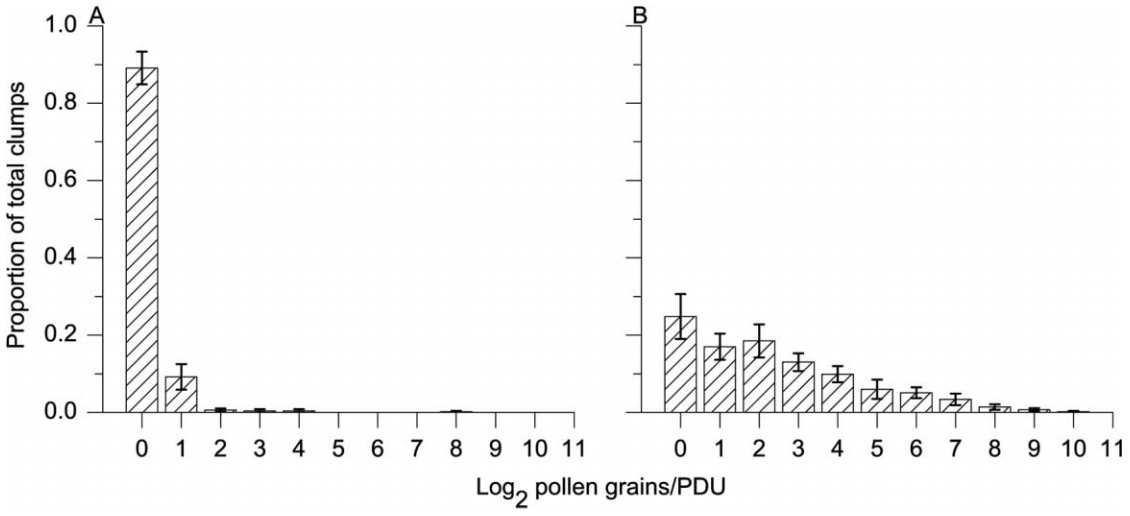

Fig. 2 Average proportion (mean ± SE) of $\log_2$(pollen dispersal unit [PDU] size) for anemophilous (*A*) and zoophilous (*B*) species studied in Brazil and presented in table 1. Bars are centered on bin lower bounds (bin width = 1).

Discussion

Our results showed that there were sufficient differences among species that a measure such as the mean $\log_2$ of PDU size could reliably differentiate pollen vectors. Not only did anemophilous species have a much higher proportion of monads than did the zoophilous species, but they also had lower means and standard deviations. The lognormal distribution provided a good description of the distribution of PDU sizes for most anemophilous and zoophilous species, but it was less effective for the distributions of PDU sizes in *Plantago lanceolata*. In almost every example where the lognormal fit was rejected, the problem was the presence of too many monads; the lognormal has a lower limit of 0, whereas the lower bound for disaggregation is 1.0. In any case, all samples were strongly right skewed, consistent with the proposed multiplicative sequence of disaggregation events.

The statistical information contained within the distribution of PDU sizes reliably discriminated between pollination vectors, and measures (mean, standard deviation, and DI) were well correlated with one another. There were, however, some problematic categorizations among anemophilous species. For instance, *Pinus tecunumanii* and *Casuarina cunninghamiana* were categorized as zoophiles on the basis of their standard deviations, indicating that it may be a less reliable measure than the mean or DI. Further, *P. lanceolata* was placed among zoophilous species, although admittedly at the periphery of the spread. *Plantago major* also occupied this intermediate space, with its mean PDU size predicting anemophily and its standard deviation and DI predicting zoophily.

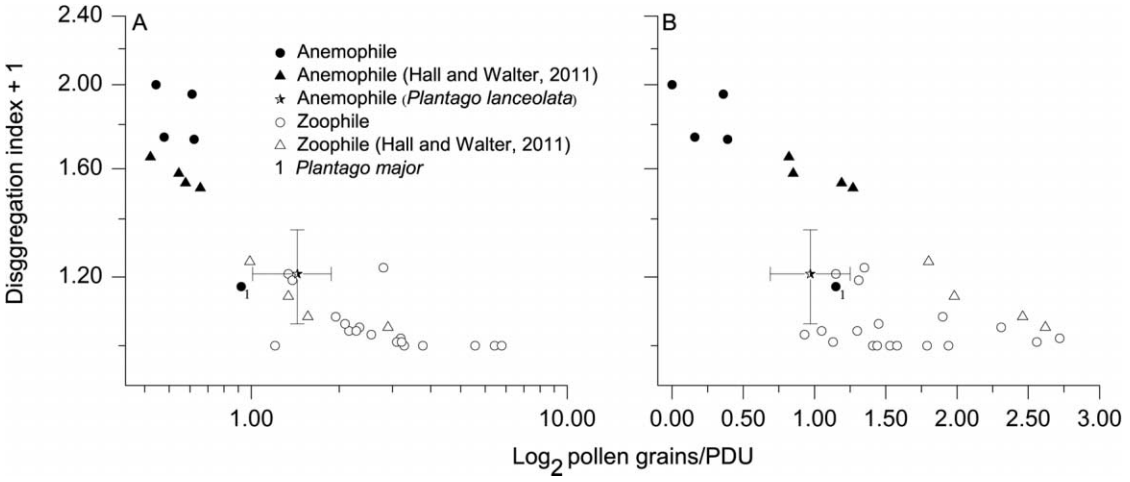

Fig. 3 Association between the disaggregation index (DI; a minimum estimate of proportion solitary grains, presented as DI + 1) and the mean of the $\log_2$(grains per pollen dispersal unit [PDU]) (*A*) and the standard deviation of the $\log_2$(grains per PDU) (*B*) for the species presented in table 1. Error bars represent the standard deviation.

In our attempt to dichotomize vectors, the ambiguous position of *Plantago* is of interest, because there are accounts of ambophily (biotic and wind pollination) in *P. lanceolata* (Clifford 1962; Stelleman 1984*b*) and in *P. major* (Kevan et al. 1993). Pollenkitt and, by extension, pollen aggregation in this species may improve the likelihood of biotic pollination, especially since their anemophilous pollination syndrome precludes some typical strategies (i.e., attractants and rewards) used by zoophilous species in biotic pollination. More specifically, in a wind-sheltered *P. lanceolata* population, insects contributed to half of the fertilization events, whereas in a windy environment, insects had a negligible contribution (Stelleman 1984*b*). Follow-up garden experiments revealed that the wind-sheltered population also had a significantly right-skewed distribution of PDU sizes, compared with windy-environment populations (Stelleman 1984*b*). This difference may represent the outcome of selection on pollen traits for improved biotic pollination. Maintaining high levels of pollen aggregation in this species may provide the flexibility to respond to changing environmental conditions (Stelleman 1984*a*). However, this point illustrates the difficulties inherent in developing a quantitative method for predicting pollination vectors. It should also be noted that there is no evidence of ambophily among the other miscategorized species or in other anemophilous species with a great deal of pollenkitt, such as *Ambrosia* sp.

A majority of the species examined fitted a lognormal distribution, which is consistent with a multistep disaggregation process. This trend was less consistent within *P. lanceolata* (i.e., 60% conformed to the lognormal distribution). A number of factors could account for the lower fit with the lognormal in this species. For example, there may have been differences between individuals in the quantity or viscosity of pollenkitt that caused variation in the distributions of PDU sizes within the anther just before release and in the overall susceptibility of PDUs to disaggregation. As we did not control for genotype, we cannot determine whether there is a genetic basis for the differences among individuals in our sample. On the one hand, Stelleman (1984*b*) found genetically based differences in pollen aggregation between two populations. In contrast, Tonsor (1985*b*) found no genetic basis for variation in gene flow distances due to differences in pollen aggregation in a comparison of 29 genotypes from the same population (e.g., the proportion of solitary monads was not significantly partially correlated with the genetic variance). Given these results, it is unclear whether genetic differences among individuals contributed significantly to the variation in our experiment. In addition, it could be that factors other than genetic variation explain our result. One possibility is that perhaps some anthers dehisced earlier and had more time for disaggregation to be initiated by drying within the anther. This phenomenon has been observed in genera such as *Actinidia*, *Erica*, *Calluna*, and *Cyclamen* (King and Ferguson 1994; Hesse 2010).

The idea that a simple measure, such as mean clump size, could reliably discriminate among vectors is tantalizing, given the cost of a careful field study to distinguish the role of pollinators versus wind. This would also be useful in studies of paleoecology, where the pollination vector is usually inferred from pollen characteristics (Hu et al. 2008; Taylor and Hu 2010). A future effort along these lines would involve more species than were used here, a standardized methodology, and a deliberate attempt to include ambophiles to ascertain whether their mean PDU size is (as with, perhaps, our *Plantago* species) indeed intermediate between anemophiles and zoophiles. A future study might also probe the development of the clump size distribution from anthesis to subsequent deposition as a function of the aggregating mechanism, disaggregating force, relative humidity, time since the onset of dehiscence, and fall time. Such a study could increase our understanding of the cases where the right-skewed PDU distributions are not lognormal as well as the sources of variation in PDU among flowers.

Acknowledgments

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Literature Cited

Ackerman JD 2000 Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. Plant Syst Evol 222:167–185.

Ackerman JD, PG Kevan 2005 Abiotic pollination. Pages 435–480 *in* A Dafni, PG Kevan, BC Husband, eds. Practical pollination biology. Enviroquest, Cambridge, ON.

Buchmann SL, JP Hurley 1978 A biophysical model for buzz pollination in angiosperms. J Theor Biol 72:639–657.

Clifford HT 1962 Insect pollination of *Plantago lanceolata* L. Nature 193:196.

Di-Giovanni F, PG Kevan, ME Nasr 1995 The variability in settling velocities of some pollen and spores. Grana 34:39–44.

Edlund AF, R Swanson, D Preuss 2004 Pollen and stigma structure and function: the role of diversity in pollination. Plant Cell 16(suppl):S84–S97.

Faegri K, L van der Pijl 1966 The principles of pollination ecology. Pergamon, Oxford.

Hall JA, GH Walter 2011 Does pollen aerodynamics correlate with pollination vector? pollen settling velocity as a test for wind versus insect pollination among cycads (Gymnospermae: Cycadaceae: Zamiaceae). Biol J Linn Soc 104:75–92.

Harder LD 1998 Pollen-size comparisons among animal-pollinated angiosperms with different pollination characteristics. Biol J Linn Soc 64:513–525.

Harder LD, SD Johnson 2008 Function and evolution of aggregated pollen in angiosperms. Int J Plant Sci 169:59–78.

Hesse M 2010 Bonding single pollen grains together: how and why? Pages 3–13 *in* J von Byern, I Grunwald, eds. Biological adhesive systems: from nature to technical and medical application. Springer, Vienna.

Hu S, DL Dilcher, DM Jarzen, DW Taylor 2008 Early steps of angiosperm-pollinator coevolution. Proc Natl Acad Sci USA 105: 240–245.

Jackson ST, ME Lyford 1999 Pollen dispersal models in Quaternary

plant ecology: assumptions, parameters, and prescriptions. Bot Rev 65:39–75.

Kevan PG, EA Tikhmenev, M Usui 1993 Insects and plants in the pollination ecology of the boreal zone. Ecol Res 8:247–267.

King JR 1981 Probability charts for decision making. Industrial, New York.

King MJ, AM Ferguson 1994 Vibratory collection of *Actinidia deliciosa* (kiwifruit) pollen. Ann Bot 74:479–482.

Lisci M, G Cardinali, E Pacini 1996 Pollen dispersal and role of pollenkitt in *Mercurialis annua* L. (Euphorbiaceae). Flora 191:385–391.

Martin MD, M Chamecki, GS Brush, C Meneveau, MB Parlange 2009 Pollen clumping and wind dispersal in an invasive angiosperm. Am J Bot 96:1703–1711.

Michalski SG, W Durka 2010 Pollen and ovule production in wind-pollinated species with special reference to *Juncus*. Plant Syst Evol 286:191–197.

Morgan MT, SCH Barrett 1990 Outcrossing rates and correlated mating within a population of *Eichhornia paniculata* (Pontederiaceae). Heredity 64:271–280.

Muller J 1979 Form and function in angiosperm pollen. Ann Mo Bot Gard 66:593–632.

Niklas KJ 1985 The aerodynamics of wind pollination. Bot Rev 51: 328–386.

Pacini E 2000 From anther and pollen ripening to pollen presentation. Plant Syst Evol 222:19–43.

Ritland K 1989 Correlated matings in the partial selfer *Mimulus guttatus*. Evolution 43:848–859.

Sabban L, N-L Jacobson, R van Hout 2012 Measurement of pollen clump release and breakup in the vicinity of ragweed (*A. confertiflora*) staminate flowers. Ecosphere 3:1–24.

Stelleman P 1984*a* Reflections on the transition from wind pollination to ambophily. Acta Bot Neerl 33:497–508.

——— 1984*b* The significance of biotic pollination in a nominally anemophilous plant: *Plantago lanceolata*. Proc K Ned Akad Wet Ser C Biol Med Sci 87:95–119.

Taylor DW, S Hu 2010 Coevolution of early angiosperms and their pollinators: evidence from pollen. Palaeontogr Abt B 283:103–135.

Tonsor SJ 1985*a* Intrapopulational variation in pollen-mediated gene flow in *Plantago lanceolata* L. Evolution 39:775–782.

——— 1985*b* Leptokurtic pollen-flow, non-leptokurtic gene-flow in a wind-pollinated herb, *Plantago lanceolata* L. Oecologia 67:442–446.

Visher GS 1969 Grain size distributions and depositional processes. J Sediment Petrol 39:1074–1106.

Willson MF 1979 Sexual selection in plants. Am Nat 113:777–790.