Functional associations of floret and inflorescence traits among grass species[1]

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The aerodynamics of wind pollination selects for an intimate relation between form and function in anemophilous plants. Inflorescence architecture and floral morphology vary extensively within the Poaceae, but the functional implication of this variation remains largely unknown. Here we quantify associations between floret, culm, and inflorescence characteristics for 25 grass species in Kananaskis, Alberta, Canada, and consider whether different architectures may implement unique mechanisms to aid pollination. The species cluster into four categories defined by all combinations of floret size (small vs. large) and inflorescence architecture (compact vs. diffuse). Species differed significantly for all 18 traits that we measured, with 12 traits differing only between floret-size classes, three differing only between inflorescence types, and three differing among both (independently or by an interaction). Based on these morphological associations, we discuss the aerodynamic and functional consequences of each category for wind pollination. The independence of inflorescence and floral traits has probably allowed exploration of all possible combinations of inflorescence architecture and floret size during the evolution of the Poaceae.

Key words: aerodynamics; floret size; inflorescence architecture; Poaceae; pollen dispersal; wind pollination.

Contrasting floral and inflorescence morphology among species of outcrossing plants commonly reflects functional differences in their interaction with pollen vectors (Grant, 1994; Harder et al., 2004). Often, this functional diversity represents adaptation to different vectors that remove, transport, and/or deliver pollen in dissimilar ways (e.g., Armbruster, 1990; Johnson et al., 1998; Castellanos et al., 2004). However, transition between pollen vectors is not the only evolutionary process responsible for reproductive diversification among flowering plants because related species with contrasting morphology often share the same vector. The grasses (Poaceae) are an obvious example with their remarkable diversity in the architecture of their inflorescences and structure of their spikelets (e.g., Figs. 1–4; Clayton and Renvoize, 1986; Doust and Kellogg, 2002), even though almost all outcrossing species are wind-pollinated. Such diversity despite a common pollen vector suggests that dissimilar reproductive morphology must allow different species to use the same vector in different ways to effect pollination.

Three studies have demonstrated that the morphology of grass inflorescences affects their interactions with airflows, thereby influencing pollination. Niklas (1987) laid the foundation for such studies with detailed observations in a wind tunnel of pollen movement around the inflorescences of a grass species with a compact panicle and another species with a diffuse panicle. Niklas found that a compact panicle acts as a bluff-bodied obstruction to airflow, so that much entrained pollen is deflected from windward stigmas and is instead captured by sedimentation from eddies on the leeward side of the inflor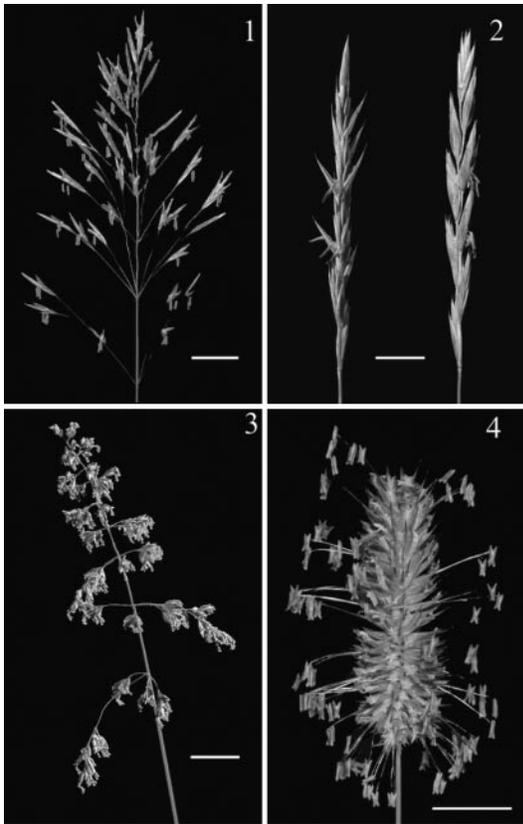escence. In contrast, a diffuse panicle disrupts airflow less, so that much pollen collides with windward-facing stigmas. Niklas (1987) also observed differences in harmonic motion between the inflorescences of the two species; the compact panicle oscillated at a higher frequency because of higher flexural rigidity of the culm. Paw U and Hotton (1989) mathematically described the relation expected between the sizes of pollen grains and the structure that interacts with the airstream to influence pollen impact with stigmas. They proposed that this interactive structure was either the inflorescence for compact inflorescences or the floret (including subtending bracts) for diffuse inflorescences. Motivated by Niklas' (1987) wind-tunnel experiments, Friedman and Harder (2004) used field manipulations to show that the oscillation of a grass inflorescence has contrasting effects on pollen removal and receipt by species with compact and diffuse panicles. Specifically, immobilization of the culms of three species with compact panicles reduced pollen removal, but did not affect pollen receipt. In contrast, immobilization did not affect pollen removal from two species with diffuse panicles, but it reduced pollen receipt. Together, these studies demonstrated that compact and diffuse inflorescences function differently to effect pollination.

Compact and diffuse panicles represent the ends of a continuum of reproductive morphologies within the Poaceae (Clayton, 1990). Grass inflorescences range from a spike-like panicle with highly contracted branches (e.g., *Phleum pratense*), to a narrow (e.g., *Phalaris arundinacea*) or broad panicle (e.g., *Deschampsia cespitosa* in Clayton and Renvoize, 1986). Furthermore, the spikelets comprising the inflorescence can vary in size and morphology. For example, the number of florets per spikelet ranges from one (e.g., *Stipa* and *Agrostis*) to at least 40 in *Eragrostis oxylepis* (Hitchcock, 1950; Clifford, 1986), with corresponding diversity in floret size. The aerodynamic consequences of floret size remain unexplored. In particular, Niklas' (1987) wind-tunnel experiments offer no insight on these effects, because both species that he studied have small florets. Thus the distinction between compact and diffuse panicles that Niklas (1987) identified may not incorporate all major features of the inflorescence and spikelet that control pollination.

Figs. 1–4.   Representative species of the four morphological classes identified by cluster analysis. **1.** *Bromus inermis*, large-flowered, diffuse inflorescence. **2.** *Elymus lanceolatus*, large-flowered, compact inflorescence, with two views of the same inflorescence. **3.** *Poa cusickii* Vasey, small-flowered, diffuse inflorescence. **4.** *Phleum pratense*, small-flowered, compact inflorescence. Scale bars = 1 cm.

A larger surface area enhances total particle capture (Niklas, 1985). However, an increase in stigma area thickens the boundary layer, which decreases the probability of pollen capture, especially at low Reynolds numbers (i.e., low wind velocity). Furthermore, the collection efficiency of an object is related to the radius of the filtering element (Vogel, 1983a). In grasses, the feather-like structure of the stigma increases surface area, while decreasing diameter, and thus ameliorates the boundary-layer problem and increases its collection efficiency (Crane, 1986).

The importance of the morphology of the receptive structure, its movement in wind, and pollen morphology for abiotic pollination suggest that traits that function together to influence pollination should be highly correlated in wind-pollinated plants. Although differences in inflorescence architecture can be characteristic of certain tribes of grasses, analysis of the phylogenetic distribution of inflorescence architecture suggests that different designs have arisen repeatedly and independently (Stebbins, 1982; Kellogg, 2000). The repeated evolution of similar architectures may reflect common selective environments and suggests that functional aspects of inflorescence architecture are subject to natural selection. In particular, selection on the functional correlation of traits can occur when certain trait combinations confer higher fitness than other combinations, given adequate genetic variance (Conner and Sterling, 1995). Because the conditions for anemophily probably require interaction and coordination between various floral, inflorescence, and culm characteristics, strong correlations among these traits could reveal associations that have evolved to optimize pollination. However, the presence of correlated traits does not necessarily mean that the correlation is selectively advantageous because trait associations can result from developmental allometry (Gould, 1996; Pigliucci et al., 1996).

In this paper, we quantify associations between floret, culm, and inflorescence characteristics for 25 grass species. We explore correlations that may be related to the aerodynamics of wind pollination and specifically consider whether different architectures possess unique mechanisms to aid pollination.

MATERIALS AND METHODS

Study site and species—We studied the grass species at two sites in each of three habitats near the University of Calgary, Barrier Lake Field Station (51°02′ N, 115°03′ W), including the understory of mixed aspen (*Populus tremuloides*)-lodgepole pine (*Pinus contorta*) forest, open meadow, and exposed windward hillside. During the 2001 flowering season, the six sites were sampled on a rotational basis during a 45-d period. At each site, we characterized the grass flora by recording the grass species in 10 plots (1 × 1 m), separated by 1 m along two 20-m transects. The origins and directions of the transects were selected randomly. A total of 25 species and subspecies were found in the transects, including *Achnatherum lemmonii* (Vasey) Barkworth, *Agropyron cristatum* (L.) Gaertn., *Agrostis scabra* Willd., *A. stolonifera* L., *Anthoxanthum nitens* (Weber) Y. Schouten and Veldkamp, *Avenula hookeri* (Scribn.) Holub, *Bromus carinatus* Hook. and Arn., *B. inermis* Leyss., *Calamagrostis rubescens* Buckley, *Deschampsia cespitosa* (L.) P. Beauv., *Elymus lanceolatus* (Scribn. and J.G. Sm.) Gould subsp. *lanceolatus*, *E. repens* (L.) Gould, *E. trachycaulus* (Link) Gould ex Shinners subsp. *trachycaulus*, *E. trachycaulus* (Pease and A.H. Moore) Cody subsp. *glaucus*, *Festuca campestris* Rydb., *F. rubra* L., *Koeleria cristata* Pers., *Leymus innovatus* (Beal) Pilg., *Muhlenbergia richardsonis* (Trin.) Rydb., *Phalaris arundinacea* L., *Phleum pratense* L., *Poa pratensis* L., *P. secunda* J. Presl. subsp. *secunda*, *P. secunda* J. Presl. subsp. *juncifolia* (Scribn.) Soreng, and *Schedonorus pratensis* (Huds.) P. Beauv. Nomenclature follows Soreng et al. (2003) and Watson and Dallwitz (2003). We measured the air velocity for 10 min twice per day on at least 30

In addition to inflorescence architecture, the efficiency of wind pollination may depend on inflorescence position and the characteristics of the supporting culm. In general, an inflorescence's aerodynamic environment depends on its height, because average horizontal wind speed increases logarithmically with height above ground level due to the presence of the boundary layer of still air near objects. The gradient in wind speed through the boundary layer depends on surface roughness, which is influenced by plant spacing and average plant height (Pasquill and Smith, 1983; Vogel, 1983a). Therefore, taller plants generally experience higher wind speeds than shorter grasses in the same habitat. Given this influence of plant form, it is not surprising that grasses generally display their florets above the boundary layer of their foliage, with the apical location of the inflorescence maximizing both the height of the structures involved in pollination (and seed dispersal) and the airspace through which the inflorescence sweeps. Indeed, for wind-pollinated species, the rate at which paternal fitness saturates (and thus the allocation of reproductive resources to male reproduction) may be determined by plant height (Burd and Allen, 1988) because pollen released from greater heights remains entrained in the airstream longer and disperses farther (Okubo and Levin, 1989).

The feather-like structure of grass stigmas has also been proposed as an adaptation that increases the efficiency of pollen capture (Faegri and van der Pijl, 1979; Whitehead, 1983).

d per habitat. Velocity was measured with a TA3 hot-wire anemometer (Air-flow Developments, Buckinghamshire, UK), at three heights above ground level (15, 30, 45 cm).

Analysis of morphological characteristics—Other than inflorescence height, all morphological traits were measured in the laboratory. After measuring the height of the bottom and top of the inflorescence for 10 individuals per species, we cut the flowering culms at ground level and placed them in vials with water. The plants were transported immediately to the laboratory (no more than 15 min) and measured.

Inflorescence architecture—Inflorescence architecture was measured using several techniques. The volume of inflorescences was determined using a Polhemus 3Space digitizer (Colchester, Vermont, USA), which measures the *x, y, z* coordinates at the tip of a clicker-pen within a $1.8 \times 1.8 \times 1.8$ m magnetic field. For each plant, we recorded the positions of nodes, lengths of branches, and the positions of the tips of the terminal florets on branches. The data were recorded directly by a laptop computer. Qhull software (Center for Computation and Visualization of Geometric Structures, version 2.6, Minneapolis, Minnesota, USA) , which implements the Quickhull algorithm (Barber et al., 1996), was used to compute the convex hull containing the set of points measured for an inflorescence. For each individual, the volume of the convex hull was computed using the same software. The length of the inflorescence was calculated from the coordinates of the most basal node that produced florets and the tip of the most distal floret using the Pythagorean theorem. The total number of florets on the inflorescence was counted and divided by inflorescence volume to calculate inflorescence density (Weller et al., 1998). Inflorescence width was measured on dried specimens collected from the field sites, except for three species, which were measured from herbarium specimens.

Culm properties—In addition to the drag created by the panicle and its mass, the oscillatory motion of grass inflorescences depends on two characteristics of the supporting culm; its length and flexural rigidity (Niklas, 1987). Flexural rigidity equals the product of the modulus of elasticity (E) and the second moment of area of the culm (I). We calculated culm elasticity based on measurements of load and displacement taken with a simple tensometer (for details see Dunn and Dabney, 1996) for the internode closest to the inflorescence. Leaf blades and the inflorescence were removed, and the length of the culm (L) was measured. In previous work, the nodes of grass species were at least as rigid and strong as the internodes (Dunn and Dabney, 1996). Culm dimensions that determine the second moments of area were measured by cutting a small cross section of the culm with a razor blade and measuring its dimensions at $10\times$ with a dissecting microscope. Because hollow culms are usually elliptical, we measured the outside dimensions of the major and minor axes (d_1 and d_2, respectively) and the inside dimensions of the major and minor axes (d_a and d_b, respectively). Based on these measurements, we calculated the second moments of area for bending parallel to the major axis (I_{maj}) and the minor axis (I_{min}) according to

$$I_{maj} = \frac{\pi}{64}(d_1^3 d_2 - d_a^3 d_b) \quad \text{and} \quad I_{min} = \frac{\pi}{64}(d_1 d_2^3 - d_a d_b^3),$$

and then averaged these values to estimate I_{ave} (Hibbeler, 2003).

Modulus of elasticity (E) was determined by loading horizontal grass culms. Each culm was placed in a protective plastic sleeve in a vise that acted as the cantilever attachment point. The initial position of the culm was recorded. A load ($P = 1.1$ g) was attached at the end of the internode segment. Deflection of the culm (Δ) was read from a grid attached to a board behind the culm. Modulus of elasticity was calculated according to

$$E = \frac{PL^3}{3I_{ave}\Delta}$$

(Finnigan and Mulhearm, 1978; Dunn and Dabney, 1996). Note that our calculated flexural rigidity (EI) probably underestimates, but is strongly correlated with, the value for an entire culm, because we measured the modulus of elasticity (E) and second moment of area (I) for the internode closest to

the inflorescence, whereas rigidity probably increases from the top to the bottom of a stem (Spatz and Speck, 2002).

Floret characteristics—To quantify pollen size and pollen production, we collected freshly dehisced anthers from 10 individuals per species, for all species in each habitat. Anthers were stored in microcentrifuge tubes with 1.0 mL of 70% ethanol. We sonicated the tubes with anthers in an ultrasonic bath to dislodge the pollen and measured the size and number of pollen grains using an Elzone 5380 particle analyzer (Micromeritics, Norcross, Georgia, USA: see Harder, 1990, for complete details).

We measured the characteristics of stigmas collected from 10 individuals per species, which were stored individually in microcentrifuge tubes with 1.0 mL of 70% ethanol. Each stigma was placed on a microscope slide, stained with 1% basic fuschin solution and photographed with a digital camera (Nikon Coolpix 4500, Tokyo, Japan) at $100\times$ magnitude under a dissecting microscope. For each photograph, we recorded the focal distance for later calculation of an image's magnification using a calibration curve derived from photographs of a micrometer scale taken at different focal lengths. The computer program Photoshop, release 6.0 (Adobe, San Jose, California, USA), was used to increase the contrast between background and object. We then used Scion Image software (Frederick, Maryland, USA) to measure stigma length and area, and the length of a stigmatic branch. If the stigma had overlapping branches, we estimated the amount of overlap and recalculated the area.

The length and width of florets and spikelets were measured with digital calipers. Dried specimens collected from the field sites were measured, except for three species from herbarium specimens.

Data analysis—We classified each species' inflorescence type according to the criteria of Hitchcock (1950) and Moss (1983). Compact inflorescences lack branches and are spike-like (e.g., Figs. 2 and 4), whereas both narrow and diffuse inflorescences bear their spikelets on branches (e.g., Figs. 1 and 3). The branches of narrow inflorescences are appressed to the inflorescence axis, resulting in a high density of florets. Diffuse inflorescences have spreading branches and a corresponding low density of florets.

Variation in characters and species was analyzed using univariate and multivariate techniques. All variables were ln-transformed due to non-normality.

We used two approaches to summarize the large matrix of correlations among variables. A variable clustering procedure (varclus procedure with centroid option, SAS, version 8.2, SAS Institute, 1999) was used to explore the relationships among variables. Varclus is a type of oblique component analysis related to multiple-group factor analysis (Harman, 1976). This procedure divides a set of numeric variables into clusters to maximize the sum across clusters of the variance of the original variables that is explained by the centroid components. We also used canonical correlation (cancorr procedure of SAS) to determine whether inflorescence characteristics, as a group, correlate with floret characteristics, as a group (Tabachnick and Fidell, 1989).

We identified groups of species with similar reproductive morphology with cluster analysis (cluster procedure of SAS). This analysis considered 18 floral, inflorescence, and culm characters for the 20 taxa for which all characters had been measured. We range-standardized the data and used mean Euclidean distance as the distance measurement (Sneath and Sokal, 1973). Clusters were identified by Ward's minimum-variance method (Ward, 1963), which combines subgroups (initially consisting of one species) at each iteration so as to minimize the within-cluster sum of squares. The number of clusters in the data was determined using the pseudo F and pseudo t^2 statistics (Milligan and Cooper, 1985). The contribution of each character to the formation of the clusters was determined using stepwise discriminant function analysis (stepdisc procedure of SAS; Tabachnick and Fidell, 1989). To illustrate the ability of the discriminant functions to separate groups, we reclassified each species using a discriminant function computed from the observations for all other species (discrim procedure of SAS).

As described below, the cluster analysis largely grouped species according to inflorescence type (compact or diffuse) and floret size (small or large). We therefore used analysis of variance (glm procedure of SAS) to identify how the 18 variables were associated with these categories. We also used multi-
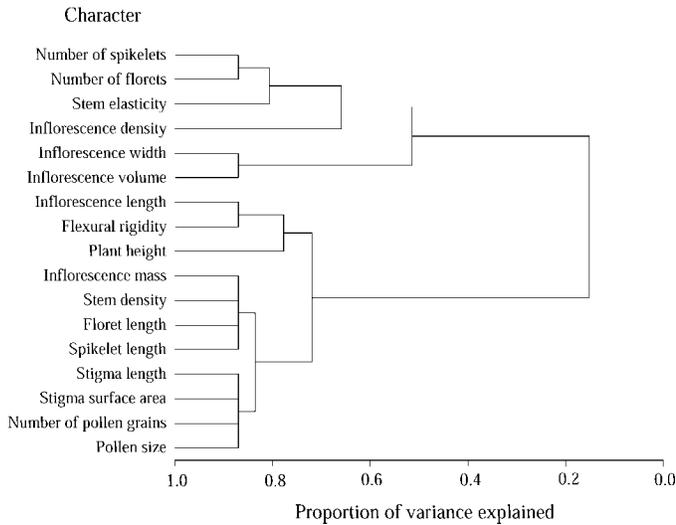
Character

Fig. 5. Cluster analysis of the correlations between the 17 variables measured. Variables clustered together have higher correlations with each other than with variables in more distant clusters.
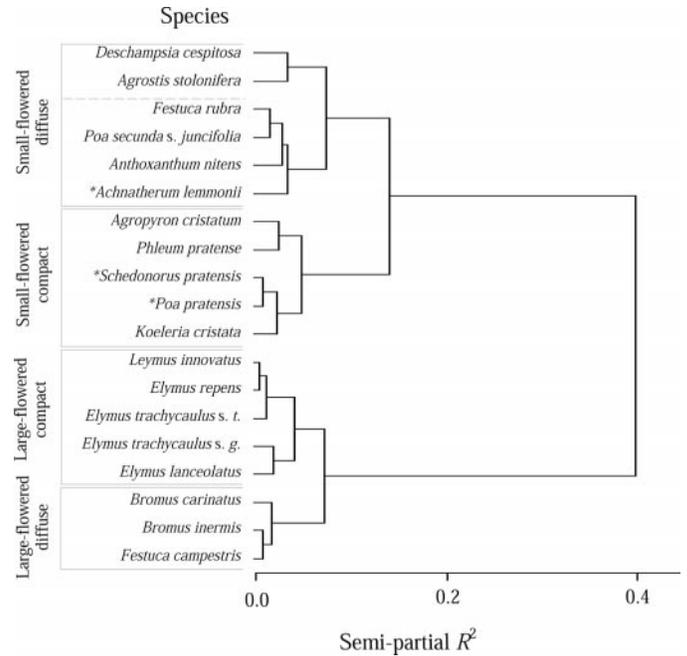

Fig. 6. Cluster analysis (Ward's method) of 20 grass species in Kananaskis, Alberta. The semi-partial R^2 scale represents the decrease in the proportion of overall variation accounted for by joining two clusters. The five significant clusters correspond largely to four classes: small-flowered, diffuse inflorescences; small-flowered, compact inflorescences; large-flowered, compact inflorescences; and large-flowered, diffuse inflorescences. * indicates exceptions to this categorization.

variate analysis of variance (Tabachnick and Fidell, 1989: glm procedure of SAS) to test for overall morphological differences between inflorescence and floret categories.

We assessed whether our observations for 21 grass species for which we had relevant data conformed to Paw U and Hotton's (1989) predictions for the optimum pollen and interactor size. Paw U and Hotton (1989) calculated the optimum pollen–interactor sizes for 28 species (including one grass species), for wind speeds 2–5 m/s (the average range they expected plants to encounter in nature). According to Paw U and Hotton's criteria, spikelet width represents the aerodynamic unit for diffuse and narrow architectures, whereas the width of the entire inflorescence represents the interactor for compact panicles. We calculated settling velocity according to Stokes' law (see Niklas, 1992). We also calculated the optimum interactor diameter for each species, given the measured range of wind speeds it experienced and its pollen size.

RESULTS

Associations between inflorescence and floret characters—
All attributes measured during this study varied extensively, with coefficients of variation among species ranging from 20% (pollen size) to 192% (inflorescence volume). Cluster analysis of all characters detected two groups of variables that correlated more strongly with each other than with variables in the other group and accounted for over 50% of the total variation in the variables (Fig. 5). One cluster included traits associated with floret size, inflorescence mass, vertical dimensions of the inflorescence, culm density, and flexural rigidity. Within this cluster of floret traits, correlations between pollen and stigma characteristics all exceed $r = 0.61$. In addition, correlations between floret length, spikelet length, inflorescence mass, and culm density are all at least $r = 0.64$. The second cluster included aspects of floret production, inflorescence size, and culm elasticity. Further splitting of these two clusters increased the proportion of variance explained only slightly.

Canonical correlation found a significant overall correlation between inflorescence characters (height, length, width, volume, number of florets, inflorescence density, inflorescence mass, flexural rigidity, and culm density) and floret characters (length of florets, stigma length, stigma-branch length, stigma surface area, pollen production, and pollen size). Two of the

six possible canonical variates differed significantly from 0 and together explained 97% of the overall variation among species (first variate $R_c = 0.99$ [86% of variance]; second variate $R_c = 0.97$ [10% of variance]). Variables in the inflorescence set that correlated strongly ($r > 0.45$) with the first canonical variate included number of florets (-0.78), flexural rigidity (0.71), inflorescence length (0.70), height (0.69), culm density (0.62), and inflorescence mass (0.59). Among the floret variables, floret length (0.92), stigma-branch length (0.63), pollen grain size (0.59), and stigma surface area (0.58) correlated with the first canonical variate. Therefore, this canonical variate largely reflects a size-number tradeoff in floret production. Indeed, multiple regression analysis found a negative relation between total floret number and floret length (partial regression coefficient, $b \pm \text{SE} = -1.30 \pm 0.182$, $t_{20} = 7.14$, $P < 0.001$), after accounting for a positive effect of inflorescence mass ($b \pm \text{SE} = 0.55 \pm 0.100$, $t_{20} = 5.47$, $P < 0.001$: overall $R^2 = 0.721$, based on ln-transformed data). In addition, the first canonical variate reveals a tendency for large-flowered species to have denser, more rigid culms. The second canonical variate largely involved inflorescence mass (0.65), culm density (0.56), and flexural rigidity (0.46) for the inflorescence variables and stigma surface area (0.56) and number of pollen grains (0.54) for the floret variables.

Associations between species—Cluster analysis of 20 species detected five clusters (Fig. 6, Table 1). These clusters do not conform to the phylogenetic relationships of these species (compare with Catalan et al., 1997; Soreng and Davis, 2000).

Based on stepwise discriminant function analysis, the variables that contributed to the formation of the two major clus-

TABLE 1. Mean $\pm$ SE of floral and inflorescence characteristics for grass (Poaceae) species in Kananaskis, Alberta within each floret-inflorescence category.

Variable	Compact influorescences		Diffuse influorescences	
	Small florets	Large florets	Small florets	Large florets
Height (cm)	46.16 $\pm$ 4.98	76.32 $\pm$ 10.61	57.49 $\pm$ 7.98	64.99 $\pm$ 1.97
Inflorescence mass (g)	0.15 $\pm$ 0.04	0.42 $\pm$ 0.07	0.21 $\pm$ 0.06	0.45 $\pm$ 0.02
Inflorescence length (cm)	5.86 $\pm$ 0.66	11.82 $\pm$ 1.39	11.34 $\pm$ 1.00	14.99 $\pm$ 2.50
Inflorescence width (cm)	1.12 $\pm$ 0.30	1.02 $\pm$ 0.18	5.27 $\pm$ 1.02	5.83 $\pm$ 0.60
Inflorescence volume (cm^3)	4.24 $\pm$ 2.19	4.10 $\pm$ 0.92	133.01 $\pm$ 60.69	119.11 $\pm$ 42.63
Elasticity (N $\cdot$ m^{-2} $\times$ 10^8)	2.87 $\pm$ 0.68	1.26 $\pm$ 0.16	2.30 $\pm$ 0.42	0.92 $\pm$ 0.012
Flexural rigidity (N $\cdot$ m^2 $\times$ 10^{-4})	0.73 $\pm$ 0.16	2.81 $\pm$ 0.75	2.05 $\pm$ 0.43	3.54 $\pm$ 1.71
Culm density (g $\cdot$ cm^{-1})	0.007 $\pm$ 0.001	0.016 $\pm$ 0.001	0.011 $\pm$ 0.002	0.016 $\pm$ 0.003
Number of spikelets	88.9 $\pm$ 19.2	19.4 $\pm$ 2.1	124.4 $\pm$ 44.9	24.2 $\pm$ 4.1
Number of florets	225.9 $\pm$ 58.4	98.5 $\pm$ 17.1	177.2 $\pm$ 35.1	134.7 $\pm$ 17.0
Inflorescence density (fl $\cdot$ cm^{-3})	262.2 $\pm$ 97.53	43.1 $\pm$ 11.72	3.57 $\pm$ 0.99	1.47 $\pm$ 0.41
Length of florets (mm)	3.85 $\pm$ 0.63	10.19 $\pm$ 0.48	4.35 $\pm$ 0.59	10.05 $\pm$ 1.03
Length of spikelets (mm)	4.89 $\pm$ 0.66	15.97 $\pm$ 0.98	6.40 $\pm$ 1.24	21.69 $\pm$ 5.96
Stigma length (mm)	1.59 $\pm$ 0.17	2.49 $\pm$ 0.28	1.33 $\pm$ 0.21	2.29 $\pm$ 0.14
Stigma-branch length (mm)	0.48 $\pm$ 0.08	0.86 $\pm$ 0.04	0.39 $\pm$ 0.05	0.89 $\pm$ 0.06
Stigma surface area (mm^2)	2.43 $\pm$ 0.61	7.15 $\pm$ 0.76	2.10 $\pm$ 0.531	7.17 $\pm$ 1.17
Number of pollen grains	7979 $\pm$ 1836	14 973 $\pm$ 3108	5028 $\pm$ 1067	17 271 $\pm$ 1047
Pollen diameter (μm)	25.47 $\pm$ 1.79	34.29 $\pm$ 2.37	25.44 $\pm$ 1.74	31.66 $\pm$ 1.40

ters included floret length, stigma-branch length, number of florets, and number of pollen grains, so that these clusters differed in floret size. The cluster of small-flowered species was further divided in two, based primarily on inflorescence density, number of florets, inflorescence volume, stigma-branch length, and floret length, producing clusters differing in architecture type. The cluster of small-flowered species with diffuse inflorescences was divided further, based on inflorescence density, inflorescence volume, length of florets, and number of florets, and so distinguished between species with many florets and those with fewer florets. Finally, the large-flowered cluster was split in two, based on inflorescence width, inflorescence density, number of florets, stigma-branch length, and length of florets. Thus, each floret-size cluster was divided into groups of species with either compact or diffuse inflorescences. Indeed, the 20 species fell into distinct groups in a simple plot
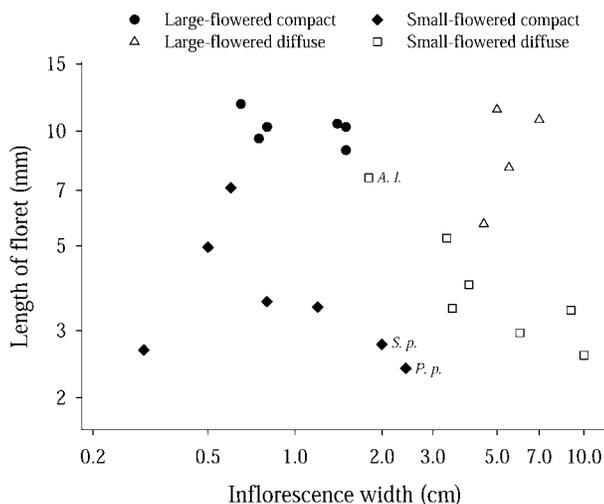

Fig. 7. Ordination of 24 grass species sampled in Kananaskis, Alberta, with respect to inflorescence width (ln-scale) and floret length (ln-scale). Different symbols illustrate the four categories of floret-inflorescence types identified by the cluster analysis. Abbreviations indicate the first letter of the genus and the species names of the exceptions to the categorization (see Fig. 6).

of inflorescence width and floret length (Fig. 7). Reclassification of each species into these categories based on discriminant functions resulted in misclassification of only two species: *Agropyron cristatum* was placed in the large-flowered compact group, and *Poa secunda* subsp. *juncifolia* was placed in the large-flowered diffuse group.

Three species did not fall clearly into the four categories based on floret size and inflorescence type. *Achnatherum lemmonii* is classified as small-flowered because it has very small stigmas and pollen (and self-fertilizes; Smith, 1944); however, based on floret length it may be better classified as large-flowered. *Schedonorus pratensis* is classified as a small-flowered species with a compact inflorescence, whereas it has a branched inflorescence. However, the inflorescence branches are appressed against the central axis of the inflorescence, resulting in a high density of florets. *Poa pratensis* was placed in a cluster of species with compact inflorescences, whereas we classified it as having a diffuse inflorescence according to the criteria of Hitchcock (1950) and Moss (1983). The architecture of *P. pratensis* inflorescences varies considerably; however, all plants tend to have a high density of florets.

Two-factor analyses of variance found contrasting patterns of variation for the 18 measured variables between classes of floret size and inflorescence type (Table 2). Only three variables (inflorescence width, volume, and density) did not differ significantly between small- and large-flowered species. In contrast, 11 variables did not differ among inflorescence types, including some unexpected traits (plant height, inflorescence mass, and culm density). Only floret number had a significant interaction between floret size and inflorescence type, with floret production differing negatively with floret size for species with compact inflorescences, but being unaffected by floret size for species with diffuse inflorescences (see Table 2). Similarly, multivariate analysis of variance found significant overall differences between floret-size classes ($F_{12,4} = 14.9$; $P < 0.01$) and inflorescence types ($F_{12,4} = 186.5$; $P < 0.001$), but no significant interaction ($F_{12,4} = 0.62$; $P > 0.5$). Therefore, these two factors seem to have largely independent influences on the reproductive characteristics of grasses.

Nearly all of the 21 species for which we measured pollen

TABLE 2.   Analyses of variance of species means for each variable considering the effects of floret type, inflorescence type, and their interaction. Letters following a test statistic for a main effect indicate significant differences, where L, S, C, or D indicate whether large- or small-flowered, compact or diffuse inflorescences, respectively, had the larger value for that trait and one, two, or three symbols indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. The significant interaction between floret type and inflorescence type for floret number resulted because small-flowered compact inflorescences produced more florets than large-flowered compact inflorescences ($t_{18} = -3.97$; $P < 0.005$), but floret production did not differ significantly with floret size for diffuse inflorescences ($t_{18} = -0.45$; $P > 0.5$).

Source of variation	Floret type	Inflorescence type	Floret × Inflorescence type
Height (cm)	$F_{1,19} = 8.12$ LL	$F_{1,19} = 0.81$	$F_{1,19} = 2.12$
Inflorescence mass (g)	$F_{1,19} = 10.52$ LL	$F_{1,19} = 1.59$	$F_{1,19} = 0.19$
Inflorescence length (cm)	$F_{1,19} = 9.40$ LL	$F_{1,19} = 14.94$ DDD	$F_{1,19} = 1.32$
Inflorescence width (cm)	$F_{1,19} = 0.05$	$F_{1,19} = 41.62$ DDD	$F_{1,19} = 1.00$
Inflorescence volume (cm³)	$F_{1,19} = 0.78$	$F_{1,19} = 50.27$ DDD	$F_{1,19} = 0.12$
Elasticity (N · m⁻²)	$F_{1,17} = 19.43$ SSS	$F_{1,17} = 1.02$	$F_{1,17} = 0.75$
Flexural rigidity (N · m²)	$F_{1,17} = 8.88$ LL	$F_{1,17} = 4.27$ C	$F_{1,17} = 2.39$
Culm density (g · cm⁻¹)	$F_{1,19} = 12.15$ LL	$F_{1,19} = 2.92$	$F_{1,19} = 0.64$
Number of spikelets	$F_{1,18} = 19.41$ SSS	$F_{1,18} = 0.13$	$F_{1,18} = 0.40$
Number of florets	$F_{1,18} = 9.77$ SS	$F_{1,18} = 0.64$	$F_{1,18} = 5.99*$
Inflorescence density (fl · cm⁻³)	$F_{1,19} = 4.07$	$F_{1,19} = 71.66$ CCC	$F_{1,19} = 0.69$
Length of florets (mm)	$F_{1,19} = 57.32$ LLL	$F_{1,19} = 3.78$	$F_{1,19} = 0.30$
Length of spikelets (mm)	$F_{1,19} = 35.36$ LLL	$F_{1,19} = 3.89$	$F_{1,19} = 0.03$
Stigma length (mm)	$F_{1,15} = 14.23$ LL	$F_{1,15} = 0.43$	$F_{1,15} = 0.18$
Stigma-branch length (mm)	$F_{1,15} = 42.44$ LLL	$F_{1,15} = 0.01$	$F_{1,15} = 0.43$
Stigma surface area (mm²)	$F_{1,15} = 31.51$ LLL	$F_{1,15} = 0.00$	$F_{1,15} = 0.05$
Number of pollen grains	$F_{1,16} = 18.76$ LLL	$F_{1,16} = 0.02$	$F_{1,16} = 1.50$
Pollen diameter (μm)	$F_{1,16} = 5.56$ L	$F_{1,16} = 0.05$	$F_{1,16} = 0.06$

characteristics have combinations of pollen and interactor size within the optimal range identified by Paw U and Hotton (1989) for wind speeds ranging from 2–5 m/s (Fig. 8). Linear regression of interactor size (D) on estimated settling velocity (V_{set}) found the following relation, $D = 0.09 + 0.20V_{set}$ ($F_{1, 24} = 8.34$; $P < 0.01$), with a slope within the range for the theoretical optimum S_o. The wind speeds experienced by species in this study fall approximately in the 2–5 m/s range, except for the species in the forest understory, which experienced lower wind speeds. For these species, the predicted interactor size is larger than expected given their pollen size.
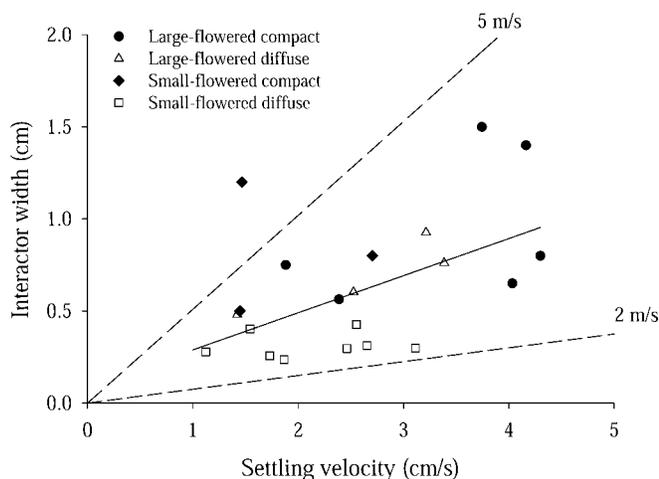

Fig. 8.   Relation between interactor size and pollen settling velocity for 21 grass species sampled in Kananaskis, Alberta. Observations plotted with reference to the predicted limits (dotted lines) of the optimum relationship for mean wind speeds of 2–5 m/s, based on Paw U and Hotton (1989). The solid line represents the best-fit linear regression. Different symbols (○, △, ◇ □) illustrate the four categories of floret-inflorescence types identified by the cluster analysis (large-flowered compact, large-flowered diffuse, small-flowered compact, small-flowered diffuse, respectively).

DISCUSSION

Our study expands upon Niklas' (1987) functional division of grasses into species with compact or diffuse inflorescences by recognizing a further, possibly functional, distinction between floret-size classes within these inflorescence types (Fig. 6). Indeed, the statistical distinction among species based on floret size is stronger (Fig. 6) and affects more reproductive traits (Table 2) than does that between inflorescence types. The finding of all possible combinations of compact and diffuse inflorescences with small and large florets and the lack of a statistical interaction between these characters (Table 2) argues that floral and inflorescence characteristics may often influence the aerodynamic environment around a plant independently. This conclusion is supported by the largely independent clusters of intercorrelated traits associated with floret size and inflorescence size (Fig. 5). The independence of inflorescence and floral traits has probably allowed exploration of all possible combinations of inflorescence architecture and floret size during the evolution of the Poaceae (see Fig. 7), resulting in the extensive diversity of reproductive morphology evident in this clade.

Possible functional differences between floret size–inflorescence type classes—Inflorescence architecture and floret size affect the general nature of a plant's aerodynamic environment in two ways. First, the extent to which an object deflects airstreams, creates leeward turbulence, and sheds vortices increases with its greatest diameter in the direction of flow (Vogel, 1983a). These effects arise primarily because larger objects retain a proportionally thicker boundary layer of relatively still air, which partially isolates them from being impacted by particles (e.g., pollen) entrained in the airstream. Consequently, large particles are more likely to collide with large objects because of their greater momentum, whereas small particles are carried by in the deflected streamlines.

The second major influence on a grass plant's aerodynamic

environment involves the oscillatory motion of inflorescences, which determines the volume of air sampled by the inflorescence and the relative airspeed near florets. The velocity and amplitude of this oscillation depends on an inflorescence's drag and mass, and the length of the subtending culm and its flexural rigidity (Finnigan and Mulhearn, 1978; Vogel, 1983a). Based on our results, inflorescence type and floret size probably affect oscillation characteristics independently. For the species that we studied, inflorescence mass varies positively with both total floret number (partial regression coefficient, $b \pm$ SE $= 1.09 \pm 0.200$, $t_{20} = 5.47$, $P < 0.001$) and floret size (floret length, $b \pm$ SE $= 1.91 \pm 0.225$, $t_{20} = 8.48$, $P < 0.001$: overall $R^2 = 0.784$, based on ln-transformed data), regardless of inflorescence type ($P > 0.5$ for main effect of type and its interactions with floret size and number). Not surprisingly, the culms of species with heavier inflorescences are also denser ($r = 0.882$, $P < 0.001$) and more rigid ($r = 0.860$, $P < 0.001$) than those with lighter inflorescences. In contrast, an inflorescence's architecture probably affects its oscillation characteristics through its influence on drag, which increases with an object's projected area (Finnigan and Mulhearn, 1978; Vogel, 1983a). However, this influence may be weaker than implied from inflorescence width measured in still air, because diffuse inflorescences deform considerably as wind velocity increases, which reduces their projected area and probably their drag (see Vogel, 1983a).

The diverse influences on the aerodynamics of wind pollination probably allow combinations of floral and inflorescence traits to result in equivalent pollination success, even though they involve contrasting mechanisms for effecting pollen removal and receipt. We now consider likely differences in the aerodynamic environments among the four classes of floret size and inflorescence type that we identified statistically and their possible effects on pollination.

A small-flowered, compact panicle functions as an integrated aerodynamic unit and oscillates in a single motion. Based on Niklas' (1987) study of such a species, *Setaria geniculata*, a single boundary layer forms around the entire inflorescence, creating downstream eddies. Pollen grains circle within these eddies and can deposit onto stigmas by sedimentation. Because of the relatively large boundary layer around the whole inflorescence, which compromises pollen removal from anthers, effective pollen release from small-flowered, compact inflorescences may often require specific mechanisms. For example, in *Phleum pratense*, the species in our study with the smallest florets and most compact panicle, the stamen filaments project horizontally and perpendicular to the inflorescence axis (Fig. 4), maximizing the distance of the anthers from the inflorescence. In contrast, the anthers of species with diffuse inflorescences (e.g., Figs. 1 and 3) and those with large-flowered compact inflorescences (e.g., Fig. 2) typically hang on lax filaments, which project out and down at angles ranging from $45° - 0°$.

A large-flowered, compact panicle should also modify the airstream as a unit; however, individual spikelets are probably large enough to create additional local airstream deflections and downstream eddies. In all species of this type that we studied, the lemma and palea spread wide open during flowering, with the stigma projecting upward between them (see Fig. 2). These bracts probably create turbulent eddies and act as scoops that direct and entrain pollen from the airstream in a manner similar to that described by Niklas and Buchmann (1985) for *Simmondsia chinensis* (Buxaceae). Linder (1991 in

Honig et al., 1992) suggested that the bracts in *Staberoha banksii* (Restionaceae) also serve this function. The greater flexural rigidity of compact inflorescences compared to diffuse inflorescences suggests that they experience less displacement. Indeed, Friedman and Harder (2004) found that compact inflorescences did not suffer reduced pollen capture when their culms were immobilized and prevented from oscillating.

Unlike the spikelets of compact inflorescences, which modify airflow communally, spikelets in diffuse inflorescences function more independently, behaving like boats moored separately in a three-dimensional stream. In diffuse panicles, spikelets oscillate relatively independently and have the potential to sweep through a large volume of air. In addition, the flexible branches of diffuse panicles allow reconfiguration of inflorescence shape as windspeed increases, reducing the inflorescence's projected width and the associated drag substantially. These features introduce alternate means of effecting pollen removal and capture.

As Niklas (1987) demonstrated for *Agrostis hiemalis*, the florets (and stigmas) of species with small-flowered, diffuse inflorescences are too small to create significant downstream eddies. As a result of the relatively thin boundary layer, pollen in anthers should be exposed to relatively high airspeeds, facilitating removal. The thin boundary layer should also allow stigmas to capture pollen almost entirely by inertial collision, as Niklas (1987) observed. However, the aerodynamic effects that Niklas observed may not be representative of all grasses with diffuse architectures, because *A. hiemalis* has very few florets (spikelets are one-flowered), clustered at the tips of branches (Hitchcock, 1950). In contrast, we found that floret size correlated negatively with the number of florets ($r = -0.79$), and that small-flowered species tend to have many crowded florets (Table 2). Such crowding could cause interference between spikelets and create very complex airflow patterns around the plant, although such effects may be ameliorated by the relatively independent oscillation of branches within the panicle, at least at low wind speeds.

In species with large-flowered diffuse inflorescences, the large spikelets (and stigmas) may create appreciable eddies and shed vortices. Vortex shedding may influence pollen removal, although this process has not been examined in detail. In addition, eddy production could direct pollen towards stigmas, allowing some pollen capture by leeward sedimentation, as well as by windward impaction, although this means of pollen capture would be compromised by erratic motion of spikelets and vortex shedding, especially at high airspeeds. Large florets, with large stigmas, will be surrounded by a thicker boundary layer and, therefore, require larger pollen with more inertia to pass through, than small-flowered species. Strong correlations of pollen size with stigma size ($r = 0.72$) and floret size ($r = 0.63$) may reflect such selection for pollen capture (also see Paw U and Hotton, 1989). It seems likely that diffuse panicles with large florets create more drag than those with small florets, resulting in greater harmonic motion, although this will be counteracted to some extent by the greater flexural rigidity of the culms of large-flowered species.

Interspecific variation in pollen and stigma characteristics—Unlike animal-pollinated species (Harder, 1998), the evolution of pollen size among wind-pollinated species seems to depend strongly on pollen-transport conditions. Pollen size in anemophilous plants probably reflects stabilizing selection that balances two conflicting influences (Whitehead, 1968;

Niklas, 1985). On one hand, small pollen has low inertia, which facilitates removal from anthers, and low settling velocity, allowing it to travel farther before falling out of the airstream, thereby increasing the probability of reaching a conspecific plant. On the other hand, large pollen has greater momentum, which increases the grain's chance of breaking away from deflected streamlines to collide with a stigma. As a result of these conflicting influences, wind-pollinated species exhibit a smaller range of pollen sizes (17–58 μm) than animal-pollinated species (5–200 μm), although average pollen size does not differ greatly among these groups (Wodehouse, 1935), contrary to popular misconception (e.g., Culley et al., 2002).

Paw U and Hotton (1989) demonstrated that variation in pollen size among wind-pollinated species (including two grasses) could be predicted by the size of the structure involved in primary interaction with airstreams (either floret or inflorescence). Their model fits our data reasonably well for the range of wind speeds that they considered (Fig. 8); however, some discrepancies arose when we considered the range of wind speeds that species actually experienced during our study. Although Paw U and Hotton's model was intended to reflect large-scale patterns of fluid flow, this inconsistency suggests that relevant features of the interacting structure vary with floret size, in addition to inflorescence architecture (compact versus diffuse inflorescences). In particular, the spreading of the lemma and palea during flowering can be especially pronounced for large-flowered species. Thus, the relevant measure of spikelet width as the obstruction to airflow is unclear, because air can be channeled through this space to facilitate pollen capture.

Our analysis detected several additional morphological associations with pollen size, which probably have functional bases. Species with large pollen tend to be tall ($r = 0.503$, $P < 0.025$), with large florets (length, $r = 0.625$, $P < 0.005$) that invest more in pollen (number of grains, $r = 0.608$, $P < 0.005$; total volume, $r = 0.874$, $P < 0.001$) and have larger stigmas (length, $r = 0.608$, $P < 0.01$; area, $r = 0.723$, $P < 0.001$; branch length, $r = 0.667$, $P < 0.005$) than species with small pollen. Tall species expose their anthers to the faster airspeeds needed to remove large grains, because windspeed increases with elevation above the ground (Campbell, 1977). The tendency of large stigmas to deflect more air would be less disadvantageous for species with large pollen, both because the higher momentum of large grains makes them more likely to break free of deflected airstreams and collide with a stigma and because the higher settling velocity of large grains increases their sedimentation in eddies on the downstream side of stigmas (see Niklas, 1988). However, the positive relation between pollen size and both the number and total volume of pollen per floret indicates that species with large pollen invest more in pollen than species with small pollen (recall that all grasses produce one ovule per ovary). This greater investment may signal compensation for lower pollination efficiency (proportion of removed pollen grains exported to stigmas) in species with large pollen, compared to those with small pollen, although this hypothesis remains to be tested. Interestingly, the anthers of grasses (and sedges: Cyperaceae) produce pollen in an unusual manner, which may elevate the threshold airspeed for pollen liberation. Throughout development, pollen grains remain in physical contact with the tapetum, so that a single layer of pollen is arranged in the anther locule (Kirpes et al., 1996), which necessarily restricts the number of pollen grains per locule. The contact between each pollen grain and the ta-

petum may limit pollen removal to wind speeds exceeding a threshold value, which favor longer dispersal distances (Harder, 2000). Additionally, this arrangement may prevent grains from dehydrating before they leave the anther.

The elaborate feathery structure of grass stigmas suggests that they are the product of selection (Whitehead, 1983; Linder, 1998). However, no studies have explored the aerodynamics of stigma morphology and the capture efficiency of different designs. The most analogous system may be silkmoth antennae, for which Vogel (1983b) found appreciable drag and downstream eddies created by the antennae. Studies of small-scale fluid dynamics around moth antennae show that the spacing and "leakiness" of the branches affect how the antennae sample their fluid environments (Koehl, 1996). These studies indicate that the branched structure of a grass stigma may increase its capture efficiency and enable air to flow through the stigma and thus filter pollen of specific sizes directly from the air (correlation between stigma length and pollen size, $r = 0.608$, $P < 0.01$). This may be especially true for stigmas of large-flowered plants, which tend to be larger and project further from floret bracts. Furthermore, stigmas of various sizes may differ in their elasticity. The branching pattern of stigmas shows considerable interspecific variation (J. Friedman, personal observation), the function of which remains unexplored.

The diversity of panicle morphologies and spikelet and floret constructions within Poaceae suggests that many alternate combinations of inflorescence and floral traits allow similar pollination success. Many of the morphological associations that we observed can be interpreted as aerodynamic adaptations. However, these interpretations largely await verification, given that the interaction of grass inflorescences and airstreams has been examined for only two species (Niklas, 1987) and that study did not consider pollen removal. Clearly, much remains unknown about the aerodynamic details of wind pollination and its consequences for morphological evolution in plants.

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