


The biomechanics of pollen release: new perspectives on the evolution of wind pollination in angiosperms

David Timerman*  and Spencer C.H. Barrett 

Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, M5S 3B2, Canada

ABSTRACT

Evolutionary transitions from animal to wind pollination have occurred repeatedly during the history of the angiosperms, but the selective mechanisms remain elusive. Here, we propose that knowledge of pollen release biomechanics is critical for understanding the ecological and evolutionary processes underpinning this shift in pollination mode. Pollen release is the critical first stage of wind pollination (anemophily) and stamen properties are therefore likely to be under strong selection early in the transition. We describe current understanding of pollen release biomechanics to provide insights on the phenotypic and ecological drivers of wind pollination. Pollen release occurs when detachment forces dominate resistive forces retaining pollen within anthers. Detachment forces can be active or passive depending on whether they require energy input from the environment. Passive release is more widespread in anemophilous species and involves processes driven by steady or unsteady aerodynamic forces or turbulence-induced vibrations that shake pollen from anthers. We review empirical and theoretical studies suggesting that stamen vibration is likely to be a key mechanism of pollen release. The vibration response is governed by morphological and biomechanical properties of stamens, which may undergo divergent selection in the presence or absence of pollinators. Resistive forces have rarely been investigated for pollen within anthers, but are probably sensitive to environmental conditions and depend on flower age, varying systematically between animal- and wind-pollinated species. Animal and wind pollination are traditionally viewed as dichotomous alternatives because they are usually associated with strikingly different pollination syndromes. But this perspective has diverted attention from subtler, continuously varying traits which mediate the fluid dynamic process of pollen release. Reinterpreting the flower as a biomechanical entity that responds to fluctuating environmental forces may provide a promising way forward. We conclude by identifying several profitable areas for future research to obtain deeper insight into the evolution of wind pollination.

Key words: animal pollination, ambophily, biomechanics, evolutionary transitions, floral traits, plant reproduction, pollen dispersal, stamens, wind pollination

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* Address for correspondence (Tel: +1 416 888 1565; E-mail: david.timerman@mail.utoronto.ca)

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I. INTRODUCTION

As pollen lacks an intrinsic dispersal mechanism it must be dispersed using agents in the environment. Early seed plants relied on wind to transport pollen to ovules (Taylor & Millay, 1979; Niklas, 1997), and this ancestral mode of pollination (anemophily) predominates in gymnosperms (Owens, Takaso & Runions, 1998; Lu *et al.*, 2011). The rise of angiosperms was associated with a transition from wind pollination to biotic pollination (zoophily) in which plants engage the services of flower-feeding animals for pollen transfer (Crane, Friis & Pedersen, 1995). It has been argued that animal pollination was favoured in angiosperms because it is less wasteful of pollen than wind pollination (Regal, 1977; Cruden, 2000). According to this view, animal vectors transport pollen directionally among flowers whereas wind transports pollen randomly due to its inherently stochastic nature. Despite these predicted differences in pollen transfer efficiency, wind pollination has evolved independently from animal pollination at least 65 times in diverse angiosperm lineages (Linder, 1998), is currently represented in approximately 10% of species (Ackerman, 2000), and occurs in every major terrestrial ecosystem, sometimes as the dominant pollination mode (Regal, 1982). The repeated evolution of anemophily and its ecological success in many environments suggests that wind pollination is a more effective means of pollination than animal pollination in some circumstances.

Darwin struggled to understand the evolution and maintenance of wind pollination in angiosperms, given its perceived inefficiency relative to animal pollination (Darwin, 1876, pp. 406–407). He thought it unlikely for wind pollination to evolve after animal pollination was established in a lineage, a view which prevailed until the latter half of the 19th century. The eventual realization that wind pollination is a derived condition in angiosperms came primarily from comparative analysis of fossils and modern relatives displaying analogous features (Hickey & Doyle, 1997; Gottsberger, 1988; Crepet *et al.*, 1991; Thien *et al.*, 2009), and through the phylogenetic reconstruction of the evolutionary history of pollination systems (Linder, 1998; Friedman & Barrett, 2008; Hu *et al.*, 2008). Although the general polarity of the evolutionary transition from animal

to wind pollination is now firmly established, with a few exceptions involving the reverse sequence (Wragg & Johnson, 2011; Huang, Xiong & Barrett, 2013), the ecological conditions and selective mechanisms favouring the transition from animal to wind pollination have remained elusive with few empirical and theoretical investigations on this subject. Our limited understanding of this transition is remarkable given that other reproductive transitions in angiosperms have attracted sustained research interest since Darwin (reviewed in Barrett, 2010), including shifts between different biotic pollen vectors (e.g. bee to bird pollination), mating systems (e.g. outcrossing to selfing) and sexual systems (e.g. hermaphroditism to dioecy).

Several authors have speculated on the environmental conditions, progenitorial forms, and selection mechanisms that might give rise to the evolution of wind pollination (Whitehead, 1969; Cox, 1991; Culley, Weller & Sakai, 2002; Friedman & Barrett, 2009). The consensus view appears to be that disruption and loss in pollinator service is probably the main factor that is ultimately responsible for driving transitions from animal to wind pollination. However, the basic premise of this hypothesis – that there is preferential selection for animal pollination because it is more efficient than wind pollination – has never been critically scrutinized. Furthermore, the same ‘reproductive assurance’ hypothesis is most often used to explain the evolution of selfing from outcrossing (Darwin, 1876, pp. 407–408; Eckert, Samis & Dart, 2006), raising the question of what circumstances might favour these quite different pollination systems.

Environmental conditions favouring wind pollination have been inferred mostly from biogeographic and habitat trends in the occurrence of wind-pollinated species (e.g. Friedman & Barrett, 2008; Rech *et al.*, 2016). Wind pollination is most common in grasslands and among the dominant species of temperate, boreal, and montane forests, and is relatively uncommon in tropical forests (Regal, 1982). It is generally considered to be most effective in communities with high conspecific plant density in which the probability of pollen dispersal among compatible mates is more likely to occur than at low density. Habitat complexity and persistent rainfall are both thought to disfavour wind pollination because both can result in the loss of pollen from airflows

(Whitehead, 1969; Regal, 1982; Di-Giovanni & Kevan, 1991; Rech *et al.*, 2016). Thus, the advantages of wind pollination have largely been inferred from ecological patterns of abundance across global ecosystems rather than through direct experimental comparisons of the efficacy of wind and animal pollination in specific ecological contexts.

For anemophily to evolve, plants must be able to release and disperse pollen in airflows and capture airborne pollen. Wind-pollinated species therefore possess a characteristic suite or syndrome of reproductive traits presumably reflecting convergent adaptation for these aerodynamic processes. Traits typically associated with wind pollination include small dry pollen grains produced in large numbers per flower, reduced corolla size, expanded stigmatic surfaces, flexible stamens, uniovulate and often unisexual flowers (see table 1 in Friedman & Barrett, 2009). The function of some anemophilous traits are understood, at least in principle, from a biomechanical perspective (see Geitmann, Niklas & Speck, 2019) developed from aerodynamic analysis (Niklas, 1985, 1992), although experimental studies testing such principles are remarkably limited (Ackerman, 2000). Some species exhibiting zoophilous traits are nonetheless capable of some degree of wind pollination, suggesting morphological or structural predisposition to the evolution of anemophily (Friedman & Barrett, 2009). Species that rely on both pollen vectors (ambophily) are uncommon and there is uncertainty as to whether they represent intermediate stages in the transition from animal to wind pollination or an evolutionarily stable state (Culley *et al.*, 2002). There have been few attempts to apply aerodynamic theory to species with these mixed pollination systems (but see Cresswell *et al.*, 2004; Timerman & Barrett, 2018), and it is often unclear which of their floral characteristics promote wind pollination.

A critical challenge for investigating the origins of wind pollination in angiosperms is the paucity of species known to exhibit intraspecific variation in pollination mode involving differentiated populations adapted to either animal or wind pollination. This contrasts with other reproductive transitions in angiosperms, including shifts between different biotic pollen vectors, as these can display distinct ecotypes or intermediate/transitional forms (Johnson, 2006). The occurrence of intraspecific variation in these systems has provided opportunities for investigating the ecological and functional mechanisms underpinning reproductive transitions and the precise sequence of adaptive changes in floral structures that result from these shifts (Barrett, 2010).

The apparent absence of intraspecific variation linking animal and wind pollination may have a biological basis associated with the nature of the transition and the maladaptiveness of transitional forms. However, it is also possible that the apparent absence of intraspecific variation is because wind pollination and ambophily have not received the same level of research interest as animal-pollinated systems. In this review, we argue that part of this neglect is because of the limited understanding of the biomechanical features of wind

pollination mechanisms. Despite some early progress on applying aerodynamic theory to flowers (Niklas, 1985, 1992), our knowledge of the physical mechanisms governing wind pollination is still rudimentary because relatively few biologists have adopted a biomechanical approach to investigating floral evolution (but see Ackerman, 2000; Vallejo-Marín, 2019). There is therefore a lack of basic information on intraspecific variation in functional traits promoting pollen release, transport and capture.

Here, we describe our current understanding of pollen release mechanisms in wind-pollinated angiosperms using a biomechanical perspective and also by drawing on relevant studies of other land plants and fungi. We have chosen to focus on the pollen release process because it is the critical first stage in wind pollination and traits governing pollen release are likely to be under strong selection during early stages in the transition from animal to wind pollination. Our primary objectives are to identify which floral traits of wind-pollinated species contribute directly to the liberation of pollen from anthers and to demonstrate how biomechanical approaches can reveal the specific functions of floral traits with respect to wind pollination. We begin with a brief overview of the structure and diversity of stamens and the meteorological correlates of pollen release. This is a natural starting point for our discussion because stamens are the organs of pollen release and this process is highly dependent on environmental conditions. We then review the range of physical mechanisms that have been observed or proposed to be involved with the release of pollen into air flows. We classify these mechanisms as active or passive depending on whether they are self-powered (active) or require energy input from the environment (passive). We discuss passive release mechanisms in greater detail focusing first on wind-induced forces which mobilize pollen grains and then on attachment forces which resist pollen release. The information on pollen release mechanisms is then used to provide insights into our understanding of the functional aspects of anemophily and factors that constrain or promote the evolution of wind pollination. We end our review by identifying several promising research directions that are likely to provide novel insights on the evolution of wind pollination.

II. STRUCTURE AND DIVERSITY OF STAMENS

Stamens are the male reproductive structures of angiosperms and thus the initiation points for the dispersal process. Although stamens come in many forms and configurations, they are fashioned on a basic ground plan consisting of two morphologically distinct parts: the filament and the anther (D'Arcy, 1996). The filament is a tubular stalk of vascular tissue which supports the anther and serves as a conduit for water and nutrients. The anther is an enlarged body attached to the tip of the filament and the site of male meiosis and pollen production (Scott, Spielman & Dickinson, 2004). On

ripening, anthers open by splitting (dehiscing) along a seam of specialized epidermal cells so that pollen can exit and participate in the pollination process (Keijzer, 1987; Pacini, 2000).

Stamens vary extensively in the mode of attachment of the anther to the filament, the size and shape of both organs, and the pattern of anther dehiscence (D'Arcy, 1996). The range of stamen diversity is functionally related to pollination mode (Endress, 1996; Bernhardt, 1996) but it seems likely that there is less structural variation among wind-pollinated species because their evolution is canalized by the underlying physical principles and constraints of wind pollination (Niklas, 1985). By contrast, in animal-pollinated taxa, stamen diversification is driven by myriad biotic interactions related to the pollination and mating systems of species. We are not aware of any studies that have verified this prediction and future comparative analyses of stamen traits of animal *versus* wind-pollinated species would seem warranted.

Typically, anthers of anemophilous species are versatile (hinged) or rigidly fixed upon the filament at their base, dehisce longitudinally or less commonly *via* apical slits, project away from the central axis of the flower on exerted filaments and produce numerous, small, dry, unsculptured pollen grains with few apertures (Hufford & Endress, 1989; Endress & Stumpf, 1991; Ackerman, 2000; Kellogg, 2015). Filaments are usually long, slender and flexible, for example in grasses, but stout filaments occur in catkin-bearing trees. It is unclear what the mechanistic basis is for most stamen characteristics associated with wind pollination and little is known about their phylogenetic histories.

III. METEOROLOGICAL DRIVERS OF POLLEN RELEASE

Most anemophilous species exhibit diurnal cycles of pollen release driven by meteorological factors (Dowding, 1987; Jackson & Lyford, 1999; Cresti & Linskens, 2000; Jones & Harrison, 2004; Dahl *et al.*, 2013). Although pollen release from stamens has rarely been quantified directly, numerous studies involving a wide variety of herbaceous and woody taxa indicate that airborne pollen concentrations over populations are greatest mid-day to late afternoon and negligible during the night (e.g. Jones, 1952; Ogden, Hayes & Raynor, 1969; Norris-Hill, 1999; Laursen *et al.*, 2007; van Hout *et al.*, 2008; Martin, Chamecki & Brush, 2010; Borycka & Kasprzyk, 2018). Meteorological measurements (e.g. relative humidity, solar radiation, temperature, and wind speed) and pollen concentration data suggest a causal relation, but disentangling the relative effects of individual meteorological variables is challenging given that they are often highly correlated in time (Martin *et al.*, 2010; Marceau *et al.*, 2011). The true nature of the relations between meteorology and pollen release mechanisms is further confounded by population attributes including population density and size, variation in reproductive investment, stochastic effects

of atmospheric transport and mixing, and sampling design [i.e. the number of sampling stations, and their three-dimensional positions relative to the population (Aylor, 2005; Vogel, Pauling & Vogel, 2008; Liu *et al.*, 2016; Wang *et al.*, 2017; Hu *et al.*, 2020)]. To our knowledge, these effects on pollen concentration have not been systematically reviewed and many open questions remain on the micrometeorology of pollen release, particularly at the scale of individual flowers and inflorescences.

Meteorological conditions during anthesis influence pollen release by initiating anther dehiscence, the dehydration-driven opening of the anther wall (Linskens & Cresti, 1988; van Hout *et al.*, 2008; Dahl *et al.*, 2013). Anther dehiscence ultimately governs when pollen is released and determines the environmental conditions encountered at the start of the dispersal process. Anther dehydration occurs either by reabsorption of moisture by vascular tissue, evaporation through epidermal stomata, or both. Reabsorption of water is an internal process regulated by the plant, and allows for greater control over the timing of pollination as it decouples anther dehiscence from meteorological conditions (Pacini, 2000). By contrast, evaporation is linked to meteorological parameters such as relative humidity, solar radiation, temperature, and wind speed, which leads to vapour pressure deficits (Linskens & Cresti, 1988; Jackson & Lyford, 1999). The sudden onset of cool, cloudy and humid conditions may temporarily arrest or even reverse dehiscence (Keijzer, Klooster & Reinders, 1996), a feature that also occurs in the anthers of some animal-pollinated species (Edwards & Jordan, 1992). These conditions often signify the onset of rain which causes washout of pollen from the air column resulting in male gamete wastage.

The evaporative mechanism of anther dehiscence is highly prevalent among wind-pollinated taxa, likely because it biases the probable wind speeds encountered to periods when relative humidity is low, and solar radiation and air temperature are high. These conditions not only promote evaporation, but correspond to periods of high turbulence and wind speed which facilitate pollen release and dispersal (Jackson & Lyford, 1999). Daily maximum wind speed and turbulence often occur mid-afternoon when pollen concentrations are greatest. Significantly, anemochorous (wind-dispersed) seeds are typically dispersed under similar conditions to pollen, presumably to maximize dispersal distances (Cousens, Dytham & Law, 2008). The dehiscence of the pericarp, which exposes seeds to wind, also depends on low relative humidity, thus promoting release during higher wind speeds which carry seeds over longer distances (Greene, 2005; Greene, Quesada & Calogeropoulos, 2008).

Variation among species in the characteristics of the daily pollen emission cycle reflects idiosyncratic effects of floral morphology and physiology, and their interactions with meteorological factors. For example, diurnal pollen concentration is bimodal in the annual herb *Ambrosia artemisiifolia* (common ragweed) owing to the particular structure of the androecium (Martin *et al.*, 2010). In this species, the filaments are fused to the corolla and the anthers are arranged

connately around a central pistillodium (a vestigial pistil). Upon dehiscence, the anthers expel much of their pollen into the central floret cavity where it is then carried away by wind (Bianchi, Schwemmin & Wagner, 1959). The pistillodium then begins elongating, sweeping residual pollen from the anthers and pushing it outside of the corolla, leading to secondary emission and thus to a bimodal distribution. However, these effects are obscured at high humidity because it delays anther dehiscence and slows pollen release leading to a monomodal distribution of pollen concentration (Martin *et al.*, 2010).

Many grasses flower over several days but have distinctive daily anthesis intervals lasting only a short while (several minutes to hours). Species often flower asynchronously within communities, thus minimizing the overlap in their emission curves (Gregory, 1973; Reddi, Reddi & Janaki, 1988). Reddi *et al.* (1988) hypothesized that sympatric species of grasses begin flowering in response to unique combinations of meteorological parameters, and that this partitioning of time may function to minimize heterospecific pollen transfer and promote reproductive isolation (e.g. Sano, 1989). Many further examples of species-specific patterns of daily pollen emission can be found in Dahl *et al.* (2013).

IV. CATEGORIES OF POLLEN RELEASE MECHANISMS

Pollen release in air can occur through active or passive mechanisms (Gregory, 1973; McCartney, 1994). Active release involves floral structures engineered to propel pollen from the anthers without assistance from external agents of the environment, and in some species active release mechanisms are also used to disperse seeds (reviewed in Sakes *et al.*, 2016). The energy required to discharge pollen in this way is stored internally in floral buds and is converted into the kinetic energy of stamens at maturity. For example, stamens of the tree *Morus alba* (white mulberry) and the perennial herb *Parietaria judaica* (spreading pellitory) develop under increasing elastic tension that, when released, results in a rapid catapulting motion that launches pollen from the anthers into the atmosphere (Taylor *et al.*, 2006; Franchi *et al.*, 2007). In *Ricinus communis* (castor bean) differential dehydration of anther cells during dehiscence causes pollen sacs to invert and revert rapidly, forcefully expelling pollen into the atmosphere (Bianchini & Pacini, 1996). In principle, active release can occur independently of wind speed and turbulence intensity because the mobilizing force is generated internally. Nevertheless, active release is sometimes triggered by environmental cues signifying conditions favourable to wind dispersal. For example, explosive pollen release in *Boehmeria caudata* (false nettle) is triggered by small minute-to-minute changes in solar radiation, relative humidity and temperature associated with brief wind gusts in the otherwise still subcanopy air of Brazilian tropical forests in

which this species occurs (Montoya-Pfeiffer *et al.*, 2016). In general, active release is uncommon in wind-pollinated angiosperms and appears to be restricted to the urticalean rosid lineage and Euphorbiaceae (Bianchini & Pacini, 1996; Taylor *et al.*, 2006; Montoya-Pfeiffer *et al.*, 2016; Pedersoli *et al.*, 2019). For this reason, we focus the remainder of our discussion on passive release mechanisms which are characteristic of most anemophilous species.

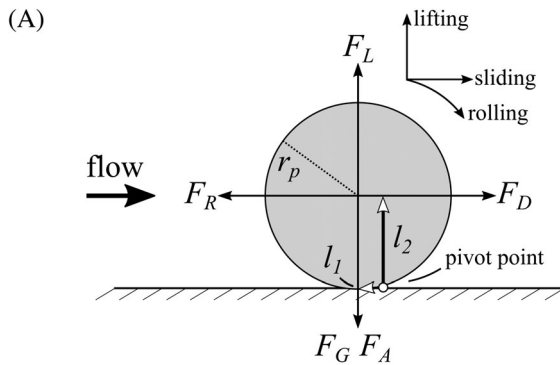
Passive release mechanisms are powered predominantly by wind energy, but gravitational settling, mechanical agitation by moving animals, and collisions with neighbouring vegetative structures may also play a role (Niklas, 1992; McCartney, 1994; Aylor, 2017). In general, the opening of anthers exposes pollen grains to the atmosphere and subjects them to a set of environmental forces. Exposure to wind can lead to their removal if the forces experienced by pollen grains exceed the strength of resistive forces keeping them attached to the anthers. Two categories of wind-induced forces may contribute to pollen release (Niklas, 1992; Urzay *et al.*, 2009; Aylor, 2017). First, aerodynamic forces act directly on pollen grains causing them to erode. These types of forces are caused by friction and pressure differences which develop over the surface of the anther as a result of air flow, and can be steady or unsteady with respect to time. Second, mechanical forces shake rather than blow pollen from anthers. They are caused by the vibrating motion of stamens and inflorescences in gusty (unsteady) conditions. The relative importance of these two components is not fully understood because pollen release is difficult to observe and quantify directly. Resolving this question is critical for determining which plant traits influence pollen release and are likely to be acted upon during selection of wind pollination from animal pollination.

V. AERODYNAMIC FORCES

(1) Conditions for pollen release

In theory, pollen release is a special case of the classical fluid dynamic problem of particle entrainment from a surface by fluid flow (Corn & Stein, 1965; O'Neill, 1968; Cleaver & Yates, 1973). The process of particle entrainment is encountered in a wide range of natural and engineering systems (e.g. spore dispersal, erosion of sand grains or soil particles, material conveyance, dust removal), and has been the subject of intensive research in diverse fields (Wang, 1990; Ziskind, 2006; Kok *et al.*, 2012; Brambilla, Speckart & Brown, 2017). A common goal in various detachment scenarios is to define the threshold conditions in which particles begin to move. Because the onset of particle motion is ultimately the result of a force imbalance, the typical approach to evaluating the limits of the equilibrium resting state involves analysing the balance of forces and moments acting on individual particles in different flow conditions.

General features of the pollen detachment and release process can be inferred by considering the aerodynamic forces acting on a scaled geometric representation of the anther in the form of a spherical particle at rest on a flat surface. It is convenient to model anthers in this way, despite variation in shape, to permit computation of closed-form solutions of fluid forces. As depicted in Fig. 1A, a particle experiences both horizontal and vertical force components of air flow, including drag (in the direction of the flow), F_D , and lift (in the direction perpendicular to the flow), F_L . Drag forces arise from fluid friction on the particle surface and pressure differences between the upstream- and -downstream-facing sides of the particle, whereas lift forces are due to pressure differences above and below the particle. Their exact formulation depends on several properties such as the size and shape of the substrate, particle diameter, atmospheric density, and wind velocity, and also whether the flow is laminar or turbulent (see Seville, Tüzün & Clift, 1997; Vogel, 1994; Gosselin, 2019).



Mode of detachment	Threshold condition
Lifting	$F_L = F_A + F_G$
Sliding	$F_D = F_R = k_s(F_A + F_G - F_L)$
Rolling	$M_D + M_L = M_A + M_G$

Fig 1. (A) Schematic of the aerodynamic and resistive forces acting on a spherical particle such as a pollen grain of radius r_p resting on a flat surface in fluid flow (thick arrow). Force vectors are depicted by thin black arrows emanating from the centre of the particle. Dislodgement of the pollen grain by lifting, sliding, or rolling (top right quadrant) occurs when the drag (F_D) and lift (F_L) forces/moments exceed the adhesion (F_A), gravitational (F_G) and frictional (F_R) forces/moments. The open circle (bottom, right of centre) represents the pivot point around which rolling occurs. Black lines terminating in an open arrow represent the lever arms, l_1 and l_2 , for rolling pollen grain removal. (B) Threshold conditions for the different modes of particle detachment from a flat surface (see text for details). F_A adhesion force; F_D drag force; F_G gravitational force; F_L lift force; F_R frictional force; k_s static coefficient of friction (depends on surface roughness of both particle and surface; see Wang, 1990); M_i the turning moment (force multiplied by lever arm, l_i) associated with force component i .

Aerodynamic forces can mobilize the particle through lifting (vertical translation), sliding (horizontal translation) or rolling (rotation) motion (see Fig. 1A), but are counteracted by the resistive forces of friction, F_R , gravity, F_G , and surface adhesion, F_A . Particle motion occurs when the sum of the mobilizing forces exceeds that of the resistive forces (see Fig. 1B for equations of incipient motion). Experiments indicate that rolling resulting from drag, rather than lift, is the most common mode of detachment for pollen-sized particles (diameter, $d_p = 10\text{--}100\ \mu\text{m}$; Cleaver & Yates, 1973; Brambilla *et al.*, 2017).

The initiation of particle motion can be expressed in terms of the minimum fluid velocity (u_i) required to rotate a particle of density $\rho_p = m_p/V_p$ and radius $r_p = d_p/2$, where m_p = particle mass, V_p = particle volume ($=4/3\ \pi r^3$). For a limiting case in which the lift and surface interaction forces are negligible (i.e. $F_L = 0$ and $F_A = 0$), the balance of moments (i.e. $M_D = M_G$, see Fig. 1B) solved for velocity gives:

$$u_i = \frac{2r_p^2 \rho_p g \tan \theta}{9\mu} \quad (1)$$

where μ is fluid viscosity, θ is the angle between the radius normal to the surface and the point of furthest contact (used in calculating moment arms for the forces), and g is the gravitational constant (Grace & Collins, 1976; Niklas, 1985; Jackson & Lyford, 1999). From Equation 1, the critical wind speed for releasing pollen grains (assuming $d_p = 10\text{--}100\ \mu\text{m}$; $\rho_p = 998\ \text{kg m}^{-3}$; $\theta = 30^\circ$; $\mu = 1.82 \times 10^{-5}\ \text{kg m}^{-1}\ \text{s}^{-1}$; see online Supporting Information, Appendix S1 for details) is expected to be on the order of $u_i \sim 10^{-1}\text{--}10^{-2}\ \text{m s}^{-1}$, which is a very low threshold. Although this suggests that pollen release is easily accomplished even under light winds, as we discuss in the next section, the formation of a boundary layer around the anther imposes an important constraint on the incipient motion of pollen grains.

According to Equation 1, the ease with which particles begin rolling depends on particle mass and size, with larger particles ($\uparrow d_p$) rolling more easily than smaller particles ($\downarrow d_p$) due to their greater drag force, and lighter particles ($\downarrow m_p$) rolling more easily than heavier particles ($\uparrow m_p$) due to their smaller weights. Producing larger pollen grains may therefore be advantageous for pollen release if achieved without concomitantly increasing mass. Some gymnosperms accomplish this by producing pollen grains with air bladders, but similar structures are lacking in angiosperms (Doyle, 2010). Reduced pollen grain size is more commonly associated with wind pollination, but is unlikely to influence the mobilization force if mass and size are coupled since they are predicted to have opposing effects on the release threshold. Data on the relation between pollen mass and size are generally lacking among wind-pollinated angiosperms, but these traits would be worthwhile investigating across multiple lineages to determine whether they have evolved in concert and there is evidence for patterns of convergence.

(2) Steady aerodynamic forces

An important constraint on pollen release is the development of a boundary layer of nearly still air around the anther. The boundary layer is a region of flow in proximity to a solid boundary in which the motion of the fluid is affected by frictional resistance exerted by the boundary (Fig. 2; Vogel, 1994; Denny, 2015). The flow within the boundary layer may be smooth and orderly (laminar) or swirling and chaotic (turbulent). Laminar flow is dominated by intermolecular viscous forces inherent to the fluid, whereas turbulence is driven by the inertia of fluid elements called eddies. The specific pattern of flow is indicated by the Reynolds number, $Re = u_\infty x / \nu$, where u_∞ is the free stream velocity of the flow, ν is the kinematic viscosity of the fluid, and x is the downstream distance from the leading edge of the surface (i.e. the edge which first meets the oncoming air). The Reynolds number is a non-dimensional quantity expressing the ratio of inertial to viscous forces within a fluid. The flow over a smooth flat plate is considered to be laminar for $Re < 10^5$ and fully turbulent for $Re > 10^7$, but small deviations in the shape of the submerged body, and the presence of surface roughness may lead to earlier onset of turbulence (Denny, 2015). Urzay *et al.* (2009) estimated $Re = 36\text{--}271$ for anthers in a sample of wind-pollinated species ($n = 13$, anther length: $L_a = 0.6\text{--}4.3$ mm; $u_\infty = 1$ m s⁻¹). Thus, the flow around anthers is expected to be laminar and smooth.

A velocity gradient establishes in the boundary layer because the layer of fluid directly in contact with the boundary ‘sticks’ and its relative velocity is therefore zero. For a stationary surface, absolute velocity increases from zero at the boundary to the velocity of the undisturbed flow (the ‘free stream’), u_∞ , some distance away (Fig. 2). Consequently, a particle embedded well in the boundary layer of the supporting substrate will experience a reduction in aerodynamic removal forces, potentially impeding dislodgement. The importance of boundary layer effects can be roughly evaluated by

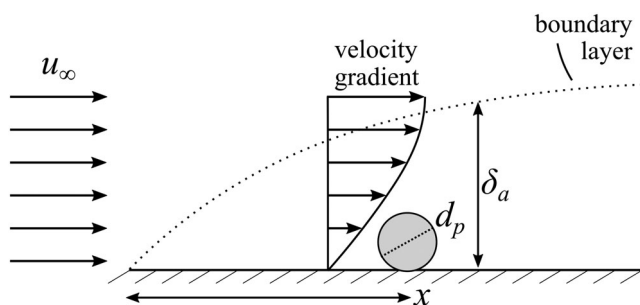


Fig 2. Sketch of a boundary layer flow over a flat surface. The dashed line indicates the thickness, δ_a , of the boundary layer as a function of distance, x , from the leading edge of the surface. A particle, such as a pollen grain, of diameter d_p embedded in boundary layer flow (i.e. $d_p < \delta_a$) experiences a reduction in flow velocity (horizontal arrows) relative to the velocity, u_∞ , of the unobstructed flow (i.e. the ‘free stream velocity’).

comparing the diameter of the particle, d_p , to the thickness of the boundary layer, δ_a , which for laminar flow is:

$$\delta_a = 5 \sqrt{\frac{\nu x}{u_\infty}} \quad (2)$$

The boundary layer becomes thinner with increasing free stream velocity, and is thicker further downstream from the leading edge. For anthers, the maximum boundary layer thickness (i.e. where $x = L_a$; see Equation 2), δ_a , is expected to be on the order of $10^1\text{--}10^2$ μm (assuming $\nu = 1.57 \times 10^{-5}$ m² s⁻¹, $x = 0.6\text{--}4.3$ mm, and $u_\infty = 1\text{--}10$ m s⁻¹, which are typical values for wind-pollinated plants; see Urzay *et al.*, 2009), which is close to the diameter (d_p) of pollen grains. All else being equal, pollen release should occur more readily in species with a greater $d_p : \delta_a$ ratio or by extension $d_p : x^{1/2}$ (see Fig. 2). In other words, minimizing anther length relative to pollen diameter should result in greater drag force on pollen grains through a reduction in boundary layer effects.

One way to potentially overcome boundary layer constraints is by increasing the ratio of $d_p : \delta_a$ by positioning anthers away from higher order boundary layers. Plants are hierarchical structures composed of modules each producing a boundary layer, one lying inside the other. For example, the anther may lie within the boundary layer of the flower, inflorescence and plant, which ultimately reside within the surface boundary layer of the earth’s surface (de Langre, 2008). By definition, wind velocity increases with height above a surface inside of a boundary layer, and the vertical velocity profile often follows a logarithmic, square-root or square function of height (Niklas, 1992). Thus, elevating the anthers above foliage potentially exposes them to higher wind speeds resulting in a thinner boundary layer around pollen grains. Probably for this reason, stamens of wind-pollinated species are commonly projected away from the central floral axis on relatively long filaments or in dangling catkins.

There have been virtually no experimental investigations of boundary layer effects on pollen release in anemophilous species, in contrast to several detailed studies of spore liberation in seedless plants and fungi. Grace & Collins (1976) found that, despite having a release threshold of only $u_r = 0.018$ m s⁻¹, the wind speed required to release 50% of the spores of the club moss *Lycopodium* from a moderately smooth surface was $u_\infty \sim 5.0\text{--}7.0$ m s⁻¹. They calculated that attaining such wind speeds near the height of strobili would require gale force winds in the lower part of the surface boundary layer. Similarly, Aylor (1978) predicted that in the maize fungal pathogen *Helminthosporium maydis*, $u_\infty = 25.0$ m s⁻¹ gale force winds would be required to achieve the experimentally determined threshold wind speed of $u_r = 5.0$ m s⁻¹. Such a high wind speed occurs too infrequently in nature to be relied upon for releasing spores. But more significantly, spore release in *H. maydis* almost always occurs under far milder atmospheric conditions (Aylor & Lukens, 1974) (see Section V.3). These results highlight the important constraining influence of the boundary layer on

spore release and raise the question of whether similar constraints also occur for anemophilous pollen release.

Angiosperm pollen may be less constrained by the boundary layer than spores. Urzay *et al.* (2009) used fluid dynamic scaling and morphological measurements from 13 wind-pollinated angiosperm species to predict the magnitude of aerodynamic forces acting on pollen grains, which were then compared to estimates of resistive force. They found that, because of the boundary layer, ambient wind speeds of $u_\infty = 1.0$ and 10.0 m s^{-1} are reduced to $u = 0.018$ and 0.041 m s^{-1} (representing a $\sim 99\%$ reduction in wind speed) in proximity to pollen grains. These local wind speeds result in steady Stokes drag forces of $F_D = 0.1\text{--}0.6 \text{ nN}$ at 1.0 m s^{-1} and $F_D = 3.9\text{--}19.7 \text{ nN}$ at 10.0 m s^{-1} (drag was calculated using the minimum and maximum trait values for the species in their sample). The resistive force was estimated using published values of pollen adhesion force measured indirectly in several buzz-pollinated angiosperm species (plants with anthers that require sonication by solitary bees to release pollen; see Vallejo-Marín, 2019), giving $F_R = 0.1\text{--}1.0 \text{ nN}$ (King & Lengoc, 1993; King & Buchmann, 1995, 1996). These estimates represent the force required to dispense pollen through small apertures located at the tips of anthers when vibrated by pollinating bees (see Vallejo-Marín, 2019). Based on these measurements, Urzay *et al.* (2009) argued that pollen release occurs in spite of the boundary layer, because the drag force roughly exceeds the adhesion force. However, pollen adhesion has never been directly measured for the anthers of anemophilous species (but see below), so the accuracy of these results cannot be evaluated. We discuss pollen adhesion in detail in Section VII.

(3) Unsteady aerodynamic forces

Unsteady aerodynamic (time-variant) forces may play an important role in overcoming boundary layer resistance. Aerodynamic forces are fundamentally transient (hence unsteady) due to the turbulent nature of the atmosphere (de Langre, 2008). The inertia of wind gusts may sweep away the relatively still air near a surface, momentarily exposing particles on the surface to the full strength of wind (Cleaver & Yates, 1973; Aylor, 1978). For example, in the fungal pathogen *H. maydis*, spore release occurred for mean wind speeds as low as $\bar{u} = 2.0 \text{ m s}^{-1}$, provided there were gusts of at least 5.0 m s^{-1} to disrupt the boundary layer (Aylor & Parlange, 1975). Significantly, this fluctuating component of wind velocity is equivalent to the measured release threshold discussed above, and the mean wind speed is far below the gale force speeds required for release under steady flow conditions.

Urzay *et al.* (2009) theoretically investigated the possibility that a similar mechanism involving unsteady aerodynamic forces may contribute to pollen release. However, the authors determined that when disrupted, the boundary layer re-emerges too quickly for the velocity fluctuation to dislodge pollen grains. Only large pollen grains ($\sim d_p > 70 \text{ }\mu\text{m}$), which are uncharacteristic of wind-pollinated species, were found to

remain outside of the boundary layer for long enough to be released. However, there is also evidence that unsteady aerodynamic forces may sometimes play a role in releasing pollen. For example, wind-tunnel analysis of *Ambrosia confertiflora* (weakleaf bur ragweed) revealed that the geometrical structure of the inflorescence results in vortex shedding (see Gosselin, 2019) which generates fluctuating regions of high shear in close proximity to pollen grains (Sabban, Jacobson & van Hout, 2012). However, it is unclear if the unsteady flow pattern contributed directly to pollen release.

VI. MECHANICAL FORCES

(1) Turbulence-induced vibration

Unsteady aerodynamic forces can also transfer energy mechanically to a system in the form of vibration. In some bryophytes and fungi, wind-induced vibration of reproductive structures is the primary mechanism of spore release. For example, wind-tunnel analysis of the moss *Atrichum undulatum* revealed that turbulent flow is required to induce seta (a stalk bearing the spore capsule) vibration, in the absence of which spores are released only in small numbers (Johansson *et al.*, 2014). Increasing turbulence intensity (i.e., the ratio of standard deviation of fluctuating wind velocity to the mean wind velocity) resulted in more vigorous vibrations and greater discharge of spores. Grace & Collins (1976) similarly demonstrated that spores of *Penicillium* are dislodged more readily in turbulent flow due to vibration of conidiophores. In laminar flow, conidiophores were deflected downwind without oscillating and thus released fewer spores. Vibration has also been shown to contribute to spreading infections of the mildew *Erysiphe graminis* in barley crops through flapping of infected leaves. Average wind speeds in closed crop canopies are typically smaller than those needed to release spores, but wind gusts within canopies can be several times greater than the local mean velocity (Aylor, 1978). Bainbridge & Legg (1976) found that wind speeds of $< 1 \text{ m s}^{-1}$ were sufficient to cause mechanical vibration of leaves capable of discharging mature conidia, whereas drag could not. Significantly, the anthers of many wind-pollinated angiosperms are borne on relatively long flexible filaments or in pendulate catkins which vibrate conspicuously in wind, leading numerous authors to speculate that vibration is also important in pollen release (e.g. Faegri & van der Pijl, 1979; Niklas, 1985; Aylor, Schultes & Shields, 2003; Pozner & Cocucci, 2006; Urzay *et al.*, 2009; Harder & Prusinkiewicz, 2013).

(2) Vibration of stamens

The mechanical structure of stamens is similar to a cantilever beam in that they are longer than wide, anchored at one end, and projected into space without additional external support. Applying a transverse load to a cantilever beam causes bending and deflection. Bending generates internal stresses which

oppose the deformation, conveying the property of elasticity, and is reversible so long as there is no permanent damage due to loading. Vibration involves cycles of bending and relaxing driven by transient or periodic loads. Natural air flows are turbulent and apply randomly fluctuating pressures/loads on the surfaces of stamens potentially causing bending and thus vibration. Pollen release may occur if the resulting inertial force (F_I) on pollen grains due to the accelerating motion of the anther overcomes the adhesion force (i.e. $F_I > F_A$).

To understand the effects of wind forcing, stamens can be modelled as a spring–mass–damper system (Urzay *et al.*, 2009), which is the classical approach to vibration analysis. In the model, the anther is likened to an inertial mass to which force is applied whereas the filament is likened to a massless spring element resisting the displacement of the anther, which is further counteracted by a damping force (see below; Fig. 3). The vibration behaviour of such a system can be characterized by two mechanical properties (reviewed in de Langre, 2019), the natural frequency (f_n) and the damping ratio (ξ), which for stamens were predicted to be on the order of $f_n = 10^1\text{--}10^2$ Hz and $\xi = 10^{-3}\text{--}10^{-2}$ (Urzay *et al.*, 2009).

Natural frequency is the rate of energy interchange between the kinetic and potential energies of a vibrating system and is related to mass, M , and spring stiffness, k , by the equation:

$$f_n = \frac{1}{2\pi} \sqrt{\frac{k}{M}} \quad (3)$$

For a cantilever beam of length L , $k = 3EI L^{-3}$, where EI is flexural rigidity, a material property quantifying resistance to bending (see Denny, 2015). Damping ratio is a measure of the energy dissipation in a dynamic system due to friction and is equal to:

$$\xi = \frac{c}{2\sqrt{kM}} \quad (4)$$

where c is the damping coefficient, a constant of proportionality relating the frictional force to the velocity of the structure.

Applying a periodic force to a cantilever beam causes it to vibrate with the same frequency as the force (see Denny, 2015; de Langre, 2019). Driving the system at its natural frequency may lead to the phenomenon of resonance which can result in abnormally large vibration amplitudes (relative to the amplitude of the exciting force). Resonance occurs because the driving force and response are in phase at f_n so the force only performs positive work thus feeding energy into the system. Damping, quantified by ξ , mitigates the resonance response by leaching energy from the system in the form of heat due to friction. Resonance occurs for damping ratios in the limits of $0 < \xi < 1$ with the response growing unbounded as $\xi \rightarrow 0$ and completely dissipating as $\xi \rightarrow 1$. The driving force for stamen motion is provided by turbulent eddies which vary in size and apply force to

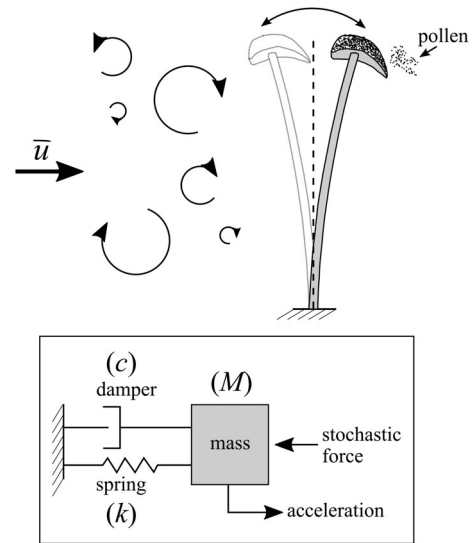


Fig 3. Top: sketch of turbulence-induced vibration of a stamen resulting in pollen release. Swirling masses of air (eddies) of different sizes and turnover frequencies (curved arrows) apply random forces to the surface of the stamen causing it to vibrate. The horizontal arrow indicates the mean component of the velocity field, \bar{u} , whereas eddies represent the fluctuating component. Adapted from Urzay *et al.* (2009). Bottom: schematic of a mass–spring–damper system used to represent stamens in vibration modelling. c is the damping coefficient, M is mass, and k is spring stiffness.

stamens at different frequencies (Urzay *et al.*, 2009). The kinetic energy of eddies scales inversely with frequency and moves from large to small scales, eventually dissipating as heat (de Langre, 2008). Given their low damping ratios, stamens should be most receptive to forcing by eddies with frequencies on the order of f_n due to resonance (Urzay *et al.*, 2009). However, because kinetic energy cascades from low to high frequencies, the magnitude of the resonance response and thus the pollen release probability should vary inversely with f_n and ξ (Timerman *et al.*, 2014b).

Several recent empirical studies have demonstrated that turbulence-driven stamen resonance is an important mechanism of pollen release in wind-pollinated angiosperms. Timerman *et al.* (2014b) used an electrodynamic shaker and high-speed video to measure f_n and ξ for stamens and quantify the effects of stamen resonance on pollen release in *Plantago lanceolata* (ribwort plantain). Both parameters fell within the theoretical ranges predicted for stamens of wind-pollinated species ($f_n = 20.0 \pm 4.0$ Hz and $\xi = 0.051 \pm 0.009$; mean \pm SD; see above) and stamen resonance resulted in a 10-fold increase in pollen release compared to vibrations at other frequencies. Pollen was released in discrete bursts, instead of a continuous stream, which suggested that pollen release is not driven by a single deterministic release threshold. Field observations of a natural population under turbulent wind conditions were consistent with these laboratory results (Timerman *et al.*, 2014b).

Stamen resonance caused rapid acceleration of anthers and was effective at releasing pollen, even at low average wind speeds ($\bar{u} < 1.25 \text{ m s}^{-1}$).

Stamen resonance driven by stamen natural frequency is the primary mechanism of pollen release among species of *Thalictrum*, a genus of herbs with multiple independent transitions between animal and wind pollination (Soza *et al.*, 2012; Soza, Haworth & Di Stilio, 2013). Wind-tunnel analysis of 36 putatively animal- and wind-pollinated species revealed, in concordance with theoretical expectations, a positive correlation between stamen acceleration and pollen release (Fig. 4D), and a negative correlation between f_n and stamen acceleration (Fig. 4C; Timerman & Barrett, 2019a), irrespective of wind speed ($\bar{u} = 0.6\text{--}2.11 \text{ m s}^{-1}$). As predicted by Equation 3, f_n was positively correlated with $L^{-3/2}$ and $M^{-1/2}$ (Fig. 4B). The range of variation among species in natural frequency and damping ratio was $f_n = 5.7\text{--}56.0 \text{ Hz}$ and $\xi = 0.02\text{--}0.21$, which is consistent with stamen resonance (Urzay *et al.*, 2009). The great majority of species analysed (91%) exhibited signatures of stamen resonance, but instances of resonance occurred less often at high values of f_n . Pollen release occurred intermittently in discrete bursts and was invariably associated with instances of stamen resonance, which occurred periodically. Thus, aerodynamic forces (i.e. drag and lift) acting directly on pollen grains did not explain pollen release in this study.

An intraspecific study involving experiments with ambophilous *T. pubescens* revealed similar trends among genotypes and heritable population differentiation in f_n ($f_n = 11.10\text{--}22.70 \text{ Hz}$; Timerman & Barrett, 2018). The variation in f_n was inversely driven by differences in stamen length (Fig. 4A, B) and was interpreted as a possible adaptive response to differences in the local pollination environment. *Thalictrum pubescens* occurs in a wide variety of habitats varying in openness, plant density and sex ratio (Timerman & Barrett, 2019b), potentially exposing populations to contrasting regimes of pollination-mediated selection. For example, insufficient pollinator service might favour genotypes with longer stamens and thus reduced f_n because it promotes more efficient pollen release in wind. To investigate the potential for this dynamic interplay, Timerman & Barrett (2018) established field arrays with screened exclosures in which they were able to manipulate the presence or absence of pollinators and measured selection on f_n . As predicted, selection under open pollination favoured greater f_n , presumably to conserve pollen for visiting insects, whereas selection favoured lower f_n under conditions of pollinator limitation. Wind speeds within the arrays were generally low ($\sim \bar{u} < 1 \text{ m s}^{-1}$), suggesting that pollinator availability has a more potent effect on selection for wind pollination than wind speed, which is contrary to the prevailing view that wind pollination requires a windy environment to function effectively. It is currently unknown if populations of other anemophilous and ambophilous species exhibit genetic differentiation in f_n or whether stamens undergo selection to promote or restrict pollen release by wind in the wild. Valuable insights would likely be obtained by quantifying

variation in the biomechanical properties of stamens in natural populations across heterogeneous habitats and establishing their relations to different abiotic and biotic components of the environment. This approach would generate data to test explicitly hypotheses on the combinations of phenotypes and environments that are favourable for wind pollination; e.g. low f_n and low pollinator availability. A more direct approach would involve the use of genetic markers to measure natural selection on stamen traits in the wild using parentage analysis (see Flanagan & Jones, 2019).

(3) Vibration of inflorescences

Vibration of larger plant structures may also contribute to pollen release. Inflorescences of wind-pollinated species exhibit remarkable structural diversity in contrast to their relatively simple floral morphologies (Harder & Prusinkiewicz, 2013). Disparity in the structural complexity of inflorescences and flowers is apparent among the inflorescences of grasses (Poaceae) and sedges (Cyperaceae) which range from spike-like panicles with highly contracted branches to narrow or broad panicles, consisting of spikelets varying in number and size (Friedman & Harder, 2005). Inflorescences and/or their supporting structures are also often flexible, and like stamens, readily vibrate in the wind. Although the aerodynamic consequences of inflorescence structure and motion have received little attention, both factors have been found to have functional roles in dispersing (see below) and capturing pollen in air (Niklas, 1987; Krick & Ackerman, 2015; McCombe & Ackerman, 2018).

Friedman & Harder (2004) investigated the role of wind-induced vibration of inflorescences for pollen release in several grass species. Experimental manipulation of grass panicle mobility and structure, through compaction and staking treatments, were used to isolate the effects of within and whole inflorescence movement. Pollen release was greatest in control groups in which mobility, and thus vibration, of inflorescences was maximized. Both inertial pollen release and floret collisions may have contributed to overcoming the resistive forces. Similar effects were reported in a study of *P. lanceolata* in which the wind-induced motion of the scape had additive effects on the acceleration of vibrating anthers (Timerman *et al.*, 2014b). However, pollen release due to stamen resonance still occurred upon immobilization of the scape. Future investigations of wind-induced vibration of pendulous catkins to determine if their motion plays an important role in inducing pollen release are needed.

VII. RESISTIVE FORCES

Resistive forces are expected to be weaker in wind-pollinated than animal-pollinated species in order to promote pollen release. Most research into pollen adhesion has focused on pollen–stigma interactions but these involve biochemical and physical mechanisms that are unique to the stigmatic

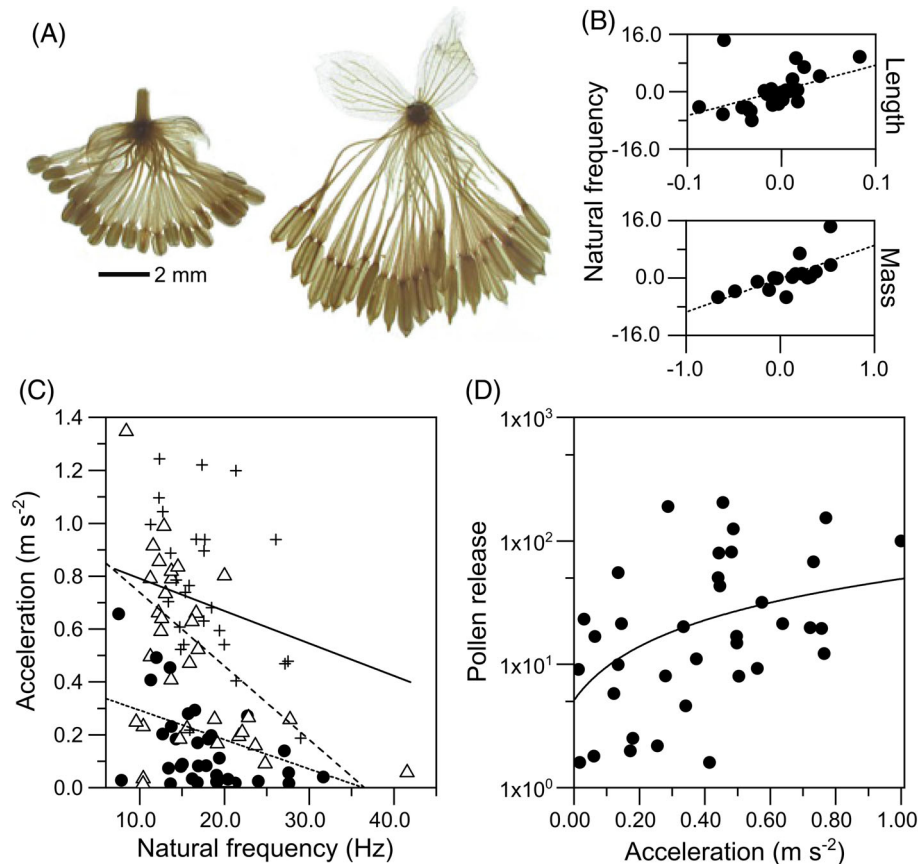


Fig 4. Analysis of vibrational pollen release for animal- and wind-pollinated species of *Thalictum*. (A) Flowers of ambophilous *T. pubescens* with stamens exhibiting high (left) and low (right) natural frequencies. (B) Correlations between phylogenetic independent contrasts of stamen natural frequency, stamen length, $L^{-3/2}$ (upper; $r^2 = 0.21$), and anther mass, $M^{-1/2}$ (lower; $r^2 = 0.55$; see Equation 3). (C) Observed (symbols) and fitted (lines) effects of stamen natural frequency on anther acceleration (root mean square) for three levels of wind speed: low (filled circles, short dashes; $r^2 = 0.24$), mid (open triangles, long dashes; $r^2 = 0.22$) and high (crosses, solid line; $r^2 = 0.20$). (D) Observed (symbols) and fitted (solid line) effects of anther acceleration on pollen release (number of pollen grains; $r^2 = 0.10$). All regression trend lines are significant at $P < 0.05$ and were modelled using linear regression (B) or phylogenetic mixed effects models (C, D) [see Timmerman & Barrett, 2019b for details]. Graphs reproduced from Timmerman & Barrett (2019b) with permission.

environment (Edlund, Swanson & Preuss, 2004). There have been far fewer investigations of pollen adhesion mechanisms, and by extension resistive forces, during the initial stage of pollination. Table 1 reports values of resistive forces measured in several animal- and wind-pollinated species using a variety of measurement techniques including vibrational (harmonic) analysis and atomic force microscopy. However, caution should be exercised in drawing general conclusions from these data given that their respective accuracies have not been systematically investigated. The most informative study to date (Lin *et al.*, 2013) measured resistive forces interspecifically for two animal- and three wind-pollinated species. Significantly, resistive forces of the animal-pollinated species far exceeded those of the wind-pollinated species. However, their lowest measurements of resistive forces were much greater than those measured in earlier studies of insect-pollinated species (King & Lengoc, 1993; King & Buchmann, 1995, 1996). A potentially confounding factor

is that the authors measured the resistive force for pollen placed on various artificial surfaces, whereas earlier investigations of pollen adhesion involved measurements of adhesion in fresh anthers. Development of a standardized protocol for measuring resistive forces and detailed uncertainty analysis of various measurement procedures would aid in advancing knowledge in this area.

(1) Adhesion theory

The force required to mobilize pollen grains ultimately depends on how strongly they adhere to the anther. Adhesion is an interfacial phenomenon in which separate material surfaces are joined by attractive/tensile forces. The main forces contributing to the adhesion of particles are Van der Waals electrostatic and liquid bridging forces (Fig. 5; Israelachvili, 2011). Van der Waals forces are a collection of short-range intermolecular forces associated with induced

Table 1. Published measurements of adhesion force for several animal- and wind-pollinated angiosperm species

Study	Species	Vector	Method	Substrate	Treatments	F_A (nN)
King & Lengoc (1993)	<i>Actinidia deliciosa</i>	animal	vib	anther	n/a	0.16
King & Buchmann (1995)	<i>Rhododendron</i> spp.	animal	vib	anther	n/a	1.50
King & Buchmann (1996)	<i>Solanum lacianatum</i>	animal	vib	anther	n/a	0.76
Thio, Lee & Meredith (2009)	<i>Ambrosia artemisiifolia</i>	wind	afm	artificial	n/a	7–13
Lin, Gomez & Meredith (2013)	<i>Helianthus annuus</i>	animal	afm	artificial	pk+	101–160
					pk–	61–70
	<i>Taraxacum officinale</i>	animal	afm	artificial	pk+	185–319
					pk–	44–50
	<i>A. artemisiifolia</i>	wind	afm	artificial	pk+	32–66
					pk–	23–31
	<i>Olea europea</i>	wind	afm	artificial	pk+	34–49
					pk–	26–30
<i>Populus nigra</i>	wind	afm	artificial	pk+	32–38	
				pk–	28–31	
Lin <i>et al.</i> (2015)	<i>H. annuus</i>	animal	afm	artificial	pk+/rh 17%	45–74
					pk+/rh 70%	90–227
					pk–/rh 17%	45–54
					pk–/rh 70%	91–199
Ito & Gorb (2019)	<i>Hypochoeris radicata</i>	animal	afm	artificial	pk+/rh <25%	35–54
					pk+/rh >75%	35–79
					pk–/rh <25%	73–111
					pk–/rh >75%	71–192

vib: vibrational analysis; afm: atomic force microscopy; pk+, pollenkitt intact; pk–, pollenkitt removed; rh, relative humidity; F_A , adhesion force, given as a range for studies involving multiple substrates.

or permanent dipoles and exist between all molecules, whereas electrostatic forces occur only between objects with charged surfaces. Liquid bridging forces arise when a liquid meniscus forms around the contact area between a particle and surface resulting in capillary and viscous forces. The relative strength of these forces depends on many factors including relative humidity (RH), surface roughness, and particle shape/size (Jones *et al.*, 2002). For pollen-sized particles ($d_p < 100 \mu\text{m}$), electrostatic forces are expected to be negligible compared to Van der Waals and liquid bridging forces (Bowling, 1988). In humid environments ($RH > 30\%$), the electrostatic force is further weakened due to the decrease in electrostatic potential, whereas the capillary force predominates over the Van der Waals forces (Bowling, 1988). Adhesive forces are diminished in dry conditions ($RH < 30\%$) because no stable meniscus can form (Jones *et al.*, 2002). The force of gravity also contributes to particle release thresholds (see Section V.1) but has been found, in pollen-

sized particles, to be up to several orders of magnitude weaker than the adhesion force (Bowling, 1988). In practice, the adhesion force is described by a distribution, often log-normal, owing to stochastic variation in the surface roughness and shapes of particles and their substratum (see Brambilla *et al.*, 2017). Therefore, anthers are likely to exhibit a distribution of pollen release thresholds rather than a single deterministic value. Significantly, the vibration experiment of Timerman *et al.* (2014b); as described in Section VI.3, revealed the existence of multiple release thresholds, given that consecutive episodes of pollen release required ever-increasing energy once a given threshold was reached.

(2) Pollen adhesion

The most important contributors to pollen adhesion in angiosperms are viscous substances (e.g. pollenkitt, tryphine, and elastoviscin) that coat the outer walls of pollen grains (Fig. 5; Hesse, 2010). These substances form liquid bridges that are substantially stronger than those produced by water, resulting in adhesion forces that are 3–6 times greater than the adhesion force of dry pollen grains (Table 1; Lin *et al.*, 2013). Pollenkitt, by far the most common of these substances, is a lipid-rich liquid derived from the breakdown of nutritive cells lining the inner chambers of the anther prior to dehiscence (Pacini & Hesse, 2005). It generally occurs in greater amounts in animal-pollinated species (e.g. Wragg & Johnson, 2011; Lin *et al.*, 2013) and facilitates adhesion to pollination vectors (Harder & Johnson, 2008). Alternatively, it may help to prevent pollen loss in wind gusts through increasing the release threshold of pollen (Timerman &

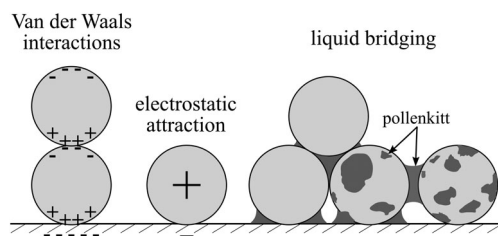


Fig 5. Schematic view of the principal mechanisms of pollen adhesion and clumping. Plus (+) and minus (–) symbols represent polarity of electric dipoles (left) or surface charges (middle).

Barrett, 2018, 2019a). Pollenkitt is less prevalent among wind-pollinated species, but is not entirely absent as is commonly perceived (e.g. Stelleman, 1984a; Lisci, Cardinali & Pacini, 1996; Lin *et al.*, 2013). A loss or reduction in pollenkitt in wind-pollinated lineages may have evolved by the relaxation of selection for sticky pollen, or alternatively, in response to selection for increased pollen release, but these hypotheses have not been tested. Interestingly, Stelleman (1984b) found that a predominantly wind-pollinated population of ambophilous *Plantago lanceolata* produced less pollenkitt than one with mixed pollination (both animal and wind), and that this difference was heritable, suggesting that the optimal production of pollenkitt may differ between the two modes of pollination.

The strength of pollen adhesion depends on the surface morphology of pollen grains and the volume and consistency of pollenkitt (Hesse, 1981; King & Lengoc, 1993; Thio *et al.*, 2009; Lin *et al.*, 2013, 2015; Ito & Gorb, 2019). The outer exine wall is not always smooth, but can be ornamented with reticulate grooves or echinate spines composed primarily of sporopollenin (Muller, 1979). Pollen surface morphology affects the adhesion force by altering the contact area between the pollen grain and anther (Lin *et al.*, 2013). Van der Waals forces scale proportionally with the tip radius of spines or the basal radius of grooves. Spines increase the spreading area of pollenkitt and thus the adhesion force, but a greater volume of pollenkitt is needed for the bridges to form, especially in species with larger spines. Wind pollination is typically associated with smooth or reticulate pollen, perhaps to reduce pollen adhesion. But this association may also arise because a simple exine structure may be aerodynamically advantageous if it reduces the terminal settling velocity of pollen grains, thus increasing the potential for dispersal (Bolick, 1990; Grega *et al.*, 2013). One exception to this pattern is the ragweed genus *Ambrosia* which produces echinate pollen as well as pollenkitt. However, *Ambrosia* species have smaller spines, less pollenkitt and a lower adhesion force than other members of their predominantly insect-pollinated family Asteraceae (Table 1; Lin *et al.*, 2013).

Liquid bridges can also form in the absence of pollenkitt due to the *adsorption* (i.e., the attachment of molecules onto a surface) of water at high relative humidity (Lin *et al.*, 2015). Thus, capillary forces may still be important for wind-pollinated species with relatively dry pollen. Significantly, pollen release generally occurs when daily relative humidity is lowest, and wind pollination is less common in persistently humid environments (Regal, 1982; Rech *et al.*, 2016). Relative humidity can also influence the adhesion properties of pollenkitt via *absorption* (i.e., the assimilation of molecules into a substance) or evaporation of water. Absorption of water at high relative humidity increases the volume of pollenkitt while reducing its viscosity through dilution, whereas evaporation of water at low relative humidity has the opposite effect. Volume and viscosity may have confounding effects on the adhesion force because an increase in volume will expand the wetted area over which liquid bridges form, but the resulting dilution may reduce their stability (Lin

et al., 2013, 2015). The sensitivity of pollen adhesion to relative humidity may also depend on pollen age, with fresh pollen being less affected than older pollen, and on the degree to which the substrate is hydrophobic or hydrophilic (Ito & Gorb, 2019). Unfortunately, the effects of relative humidity on pollen adhesion have only been explored for pollen grains on artificial surfaces and not for pollen in anthers, and few studies have systematically investigated the effects of flower age. Provided there is a vapour pressure deficit in the vicinity of the anther, evaporation should reduce adhesion over time. For example, in the buzz-pollinated kiwi fruit *Actinidia deliciosa*, pollen grains became less clumped and sticky with time since dehiscence likely due to evaporation (King & Ferguson, 1994). Drying of pollen may facilitate facultative wind pollination in pollinator-limited ambophilous populations by reducing the release threshold of pollen, but the occurrence of this phenomenon and its potential influence on reproductive fitness are unknown.

(3) Pollen clumping

Pollen grains do not occur as isolated units on surfaces but as complex multilayered structures consisting of hundreds to thousands of particles in anthers. When granular particles are brought into contact, they are subject to cohesive interparticle forces, which are similar to the adhesion forces (Fig. 5). Although little is known about interparticle forces on pollen grains, the force required to separate two spherical particles is generally expected to increase with particle diameter due to their greater contact area (Bowling, 1988). Because of natural variation in pollen grain size, the distribution of interparticle forces within a mass of pollen will be non-uniform because the cohesion force is weaker between small particles than between large particles (Henry & Minier, 2014). Heterogeneity of the cohesion force may facilitate the release of pollen clumps if contact between pollen grains is broken more readily in regions of lower adhesion. Clumping may also help to facilitate the release of a greater number of pollen grains, given that most particles within a multilayer are sheltered from wind or obstructed from moving by other particles.

Pollen clumping is less prevalent, but not absent (e.g. Martin *et al.*, 2009; Hall & Walter, 2011; Huang *et al.*, 2013; Timerman *et al.*, 2014a), in wind-pollinated species because they tend to produce less/no pollenkitt (Pacini & Hesse, 2005). Pollen clumping may be disadvantageous in wind pollination because greater force is needed to mobilize clumps than solitary grains due to their greater masses. But as discussed earlier, the gravitational force may be weaker than the adhesion (or cohesion) force by many orders of magnitude and the increased weight of clumps may have little effect on the release threshold. Clumping may in fact promote pollen release in vibrating systems due to the greater inertia of clumps than solitary grains or by increasing the drag force acting on pollen. It has also been argued that clumping may be less prevalent among wind-pollinated taxa because clumps sink faster in air than solitary grains, thus restricting dispersal distances (Hall &

Walter, 2011). However, studies of *Ambrosia* spp. demonstrated that clumps begin disaggregating immediately upon release into turbulent flow due to shear stress (Martin *et al.*, 2009; Sabban *et al.*, 2012). Indeed, Martin *et al.* (2009) reported that this disaggregation process in *A. artemisiifolia* results in a bimodal distribution of pollen dispersal distances in which larger clumps fall closer to the parent and disaggregated clumps disperse much further away. It is therefore conceivable that variation in clumping in this species may function as a form of bet hedging (see Simons, 2011) by promoting reproduction with distant, genetically unrelated mates while also simultaneously assuring reproduction by dispersing pollen to a more local and thus reliable pool of mates.

VIII. DEVIATIONS FROM MODEL ASSUMPTIONS

Much of our understanding of pollen release mechanisms is based on representations involving spherical particles on smooth flat surfaces. Pollen grains are usually not perfectly spherical, but rather exhibit a range of shapes from spheroidal to triangular, and both anthers and pollen exhibit some degree of surface roughness (Wortley *et al.*, 2015). The shape, diameter and density of pollen grains also depends on their hydration status, which may change throughout ontogeny as part of a developmental program, or in response to environmental conditions (Franchi *et al.*, 2002). Non-spherical particles lack the radial symmetry of a sphere, and the forces acting upon them (both mobilizing and resistive) depend on the orientation of the particle with respect to the underlying surface and the direction of air flow. Although much is known about the dynamics of rigid non-spherical particles once entrained in turbulent flow, the release dynamics of non-spherical biological particles has seldom been investigated in detail. Clumped pollen further complicates modelling of pollen release because clumps tend to have highly irregular shapes which can rotate, fold and break apart (Sabban *et al.*, 2012). Surface roughness in the form of small irregularities on the lining of the anther lumen are also likely to influence the adhesion force, as well as the force balance equations in Fig. 1B, through their effects on the friction coefficient for sliding and moment of torque for rolling (reviewed in Brambilla *et al.*, 2017). Future efforts to understand the biomechanics of pollen release should account for these additional complexities.

IX. POLLEN RELEASE, FLORAL TRAIT EVOLUTION AND POLLINATION MODE

Given their divergent pollination syndromes, animal and wind pollination are usually characterized in biology textbooks as dichotomous alternatives rather than suites of quantitative traits. However, the physical process of pollen release occurs at the scale of individual stamens and largely depends on their structural and adhesion properties. Traditional

pollination syndromes (Faegri & van der Pijl, 1979) may in fact provide limited information on the ability of some species to release pollen in air. For example, Timerman & Barrett (2019a) found no relation between ordination scores for floral trait data and experimental measurements of pollen release probability in their wind tunnel study of animal- and wind-pollinated *Thalictrum* species described above (see Section VI.2). By contrast, they found that pollen release probability was strongly associated with the natural frequency and damping ratios of stamens (Fig. 4). Several other species, such as rapeseed *Brassica napus* (Cresswell *et al.*, 2004), spiny madwort *Hornathophylla spinosa* (Gomez & Zamora, 1996), and *Linanthus (Leptosiphon) parviflorus* (Goodwillie, 1999), which appear to be typically zoophilous, have also been found to release pollen readily in air and thus may be ambophilous, but the factors mediating this release were not evaluated.

Apparent reversions from wind pollination to animal pollination or ambophily [e.g. *Cyperus* spp. (Wragg & Johnson, 2011) and *Plantago lanceolata* (Stelleman, 1984a), respectively] involve very limited recapitulation of the ancestral pollination syndrome (Barrett, 2013), but nevertheless have resulted in a reduced capacity for pollen release. Explaining these anomalies requires adopting a subtler perspective on wind pollination that focuses on the evolutionary dynamics of functional traits within lineages rather than on binary character state transitions among them. As we have discussed, variation in stamen traits such as filament length, anther mass, and by extension natural frequency, has direct implications for the character of pollen release and thus pollination mode. This variation is likely to represent an important target of selection for microevolutionary adaptation to local environmental and demographic conditions. Modifications to the biomechanical properties of flowers often occur at too fine a scale to capture the attention of most evolutionary biologists focusing on phenotypic traits, but this cryptic variation is likely the initial driving force underpinning the early stages in shifts of pollination mode.

X. FUTURE DIRECTIONS

Our review focused on the biomechanics of pollen release from the stamens of angiosperms and highlighted the biological and physical processes involved. This is an area that has received little attention from ecologists and evolutionary biologists and there is a paucity of comparative and experimental data on wind pollination mechanisms in angiosperms, despite their direct relevance to reproductive success and plant fitness. Below we identify four research areas that we believe will provide fertile opportunities to obtain deeper insights into the evolution of wind pollination.

- (1) Functional analysis of reproductive structures to understand better their respective roles in promoting or hindering pollen release including evaluation of: (a)

stamen traits influencing aerodynamic and mechanical forces such as damping ratio, natural frequency, pollen diameter, and Reynolds number; (b) the adhesion and cohesion of pollen within dehisced anthers to determine the minimum force required to detach individual and clumped pollen grains, and how these forces vary among anthers differing in structure, developmental age and micrometeorological environment; and (c) other reproductive traits relating to the micrometeorological environment surrounding the stamen and the bulk movement of the plant such as corolla properties, flower height, and inflorescence/catkin architecture.

- (2) Investigation of geographical variation and divergent selection of floral traits associated with pollen release, particularly for ambophilous species, across abiotic and biotic environmental gradients to determine the extent to which populations are differentiated with respect to pollen release and the ecological correlates of this variation. In particular, is there evidence for genetically based population differentiation in pollination mode with respect to anemophilous and ambophilous species? If not, what factors might account for this 'missing' intraspecific variation which commonly occurs in other reproduction transitions in flowering plants?
- (3) Examination of the biological and physical factors influencing the timing and meteorological conditions for pollen release and subsequent transport. Studies of the proximate causes and schedule of pollen release are required to determine their influence on pollen dispersal distance, mating system and fitness through paternal function, and whether wind-pollinated species use bet-hedging strategies involving local and long-distance dispersal or other pollen dispersal strategies for maximizing fitness.
- (4) Comparative and phylogenetic analysis of the evolutionary histories of stamen and inflorescence traits governing fluid flow, vibrational motion and pollen adhesion. Studies of trait evolution in wind-pollinated plants are necessary to understand better the evolutionary relationships among traits modulating pollen release, particularly whether they evolve independently or in concert, through parallel and convergent evolution, and in response to shifts in the abiotic and/or biotic environment.

We are confident that addressing these areas of inquiry through theoretical, experimental and comparative analyses of diverse animal- and wind-pollinated species will provide a fruitful future research agenda and a sound framework for integrating biomechanical principles into floral function and evolution.

XI. CONCLUSIONS

- (1) Pollen release is the critical first stage in the evolution of wind pollination from animal pollination. But it

remains unclear which particular floral traits initiate this process and under what ecological conditions they are selected. We contend that knowledge of floral biomechanics is key to addressing these issues.

- (2) Pollen release occurs when detachment forces due to wind overcome the resistive forces retaining pollen within anthers. Pollen adhesion is the primary retention mechanism and is caused by capillary, electrostatic, and Van der Waals forces. These forces have rarely been investigated for pollen within anthers, but are likely to be sensitive to environmental conditions and flower age.
- (3) There are potentially two principal detachment mechanisms involving either aerodynamic forces impinging directly on pollen grains, or mechanical forces causing vibration of anthers. Determining the relative importance of these mechanisms is crucial because they involve different features of floral and inflorescence structure.
- (4) Pollen release mechanisms may be susceptible to micro-evolutionary change linked to the prevailing pollination environment and mediated through stamen traits. In ambophilous species, divergent selection on the biomechanical properties of stamens may be especially sensitive to local pollinator availability, potentially resulting in population differentiation of floral traits.
- (5) Animal and wind pollination are traditionally viewed as dichotomous alternatives because they are usually associated with strikingly different pollination syndromes. We argue that this perspective has deflected attention from quantitative reproductive traits which play a crucial role in mediating the fluid dynamic process of pollen release and may play a key role in initiating evolutionary transitions from animal to wind pollination.

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XIV. Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Appendix S1 Explanation of parameterization used in solving Equation 1.

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