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Key words: *Arabidopsis*, fumonisin B1 (FB1), long chain base (LCB), MPK6, programmed cell death (PCD), sphingolipids.

Gone with the wind: understanding evolutionary transitions between wind and animal pollination in the angiosperms

Flowering plants exhibit spectacular diversity in flower design and display, and much of the functional basis of this variation is associated with the evolution of pollination systems. Transitions between different pollination systems among closely related species can shed light on the key characters involved in facilitating and responding to the shift. In contrast to well-studied transitions such as bee to bird pollination, we are just beginning to understand the transition from insect to wind pollination (Friedman & Barrett, 2009). The evolution of wind pollination from animal pollinated ancestors in the angiosperms is fairly common, occurring at least 65 times (Linder, 1998), and is sometimes considered irreversible (Cox, 1991; Dodd *et al.*, 1999). Nonetheless, there are some examples of reversals from wind pollination to insect pollination in flowering plants. Although these are remarkably rare, they are important because they expose key characters that may have been involved in the early radiation of the flowering plants as they co-evolved with their animal pollinators. In one of the most convincing examinations of the evolution of animal pollination from wind pollination in the predominantly wind-pollinated sedges (Cyperaceae), Wragg & Johnson (pp. 1128–1140) in this issue of *New Phytologist*, describe differences in color and scent between closely related species that are, and are not, visited by insects.

> *'… under the appropriate ecological conditions, small changes might be sufficient to achieve pollination transitions.'*

In some wind-pollinated species, the presence of insects on inflorescences is simply opportunistic (e.g. the frequent observation of pollen collecting insects on *Plantago lanceolata* and many grasses), and it is unclear in these cases how much pollen is actually transported by insects between plants. Wragg & Johnson conducted detailed and thorough experiments on putatively insect-pollinated *Cyperus* species and their closest co-occurring wind-pollinated sister species.

Their experiments included: measures of pollen removal, deposition and seed set in insect and wind exclusion experiments; pollinator observations; examining pollen motility in a wind tunnel; characterizing flower color, scent and pollen characteristics; and conducting choice experiments with artificial flowers matching these color and scent profiles. Their results and conclusions leave little doubt that pollen-collecting bees, flies and beetles play an important functional role in the pollination of the focal species *Cyperus obtusiflorus* and *C. sphaerocephalus.*

Pollination-mediated selection can create floral diversity, and although floral characters are considerably labile, diversification is never unconstrained. Historical constraint might contribute to the paucity of reversals from moth, bird or wind pollination back to bee and generalist insect pollination (discussed in Harder & Johnson, 2009). Wind-pollinated plants tend to have small flowers, with highly reduced or no perianth parts. Nectaries and scent are usually absent (Faegri & van der Pijl, 1979). Reacquiring these traits may be very difficult and insects are unlikely to reliably visit plants without some attractants. In *Dichromena ciliata*, another putatively insect-pollinated species in the Cyperaceae, white leaves and bracts surround the condensed spikelets creating a simulated simple white flower, which presumably acts to attract insects (Fig. 1; Leppik, 1955). Wragg & Johnson use an experimental comparative approach with related *Cyperus* species to demonstrate that shifts from greenish brown floral bracts to more showy white or yellow bracts, and the production of floral scent, are associated with a transition from wind to insect pollination. The morphological similarity of the insect- and wind-pollinated species (see Fig. 1 in Wragg & Johnson) suggest that, under the appropriate ecological conditions, small changes might be sufficient to achieve pollination transitions.

Fig. 1 *Dichromena ciliata* (Cyperaceae), an insect-pollinated species in the predominantly wind-pollinated sedges, with white involucral leaves surrounding the spikelets (Leppik, 1955). Photograph courtesy of Spencer C. H. Barrett.

Other work on shifts in pollination system, particularly bee to bird and bird to moth pollination, have demonstrated both the simplicity and complexity that underlie the morphological differences between species. In studies of the bee-pollinated *Mimulus lewisii* and the hummingbird-pollinated *M. cardinalis,* researchers have demonstrated that a modest number of major genes control the characters that most influence pollinator visitation rates, and that flower color is the key distinguishing feature (Bradshaw & Schemske, 2003). Others have examined the flowers of *Aquilegia formosa* (hummingbird pollinated) and *A. pubescens* (hawkmoth pollinated) and how they effect pollinator visitation and pollen transfer. Both flower orientation and color affect pollination, and the traits appear to be genetically integrated (Hodges *et al.*, 2002). Using these kinds of experiments on closely related wind- and insect-pollinated species would complement our understanding of pollination transitions. However, the challenge will be to find closely related species pairs that are animal versus wind pollinated that are interfertile and thus amenable to genetic analysis.

Indeed, empirical studies on the transition between animal and wind pollination have been hampered by the limited number of taxa containing interspecific, and especially intraspecific, variation in pollination systems. To my knowledge no species is known that shows ‘pollination ecotypes’ involving populations that are, on the one hand exclusively animal pollinated, and on the other hand, populations that are primarily wind pollinated. Early progress in understanding the selective forces leading to wind pollination was made by studies of *Thalictrum* (Ranunculaceae) (e.g. Kaplan & Mulcahy, 1971). This genus, with its range of sexual systems and both animal and wind pollination, may be a profitable group in which to explore the microevolutionary forces leading to the evolution of wind pollination. Current research on transitions between animal and wind pollination in *Schiedea* (Caryophyllaceae) in the Hawaiian Islands appears to be the best contemporary case study on the evolution of wind pollination (e.g. Golonka *et al.*, 2005; Weller *et al.*, 2006). The finding of interspecific variation in pollination system in *Cyperus* provides an alternate avenue to look at the evolutionary transition in the opposite direction.

Transitions in either direction between animal and wind pollination might involve the use of both animal and wind pollinators, either sequentially or simultaneously (Stelleman, 1984). The prevalence of this mixed pollination system, known as ambophily, is not well documented. Ambophily occurs in families such as the Salicaceae and Arecaceae (reviewed in Culley *et al.*, 2002) and involve generalist pollinators, but further work is needed to establish its frequency and the selective mechanisms that maintain ambophily. Theoretical and empirical studies are needed to explore whether ambophily is a stable strategy selected for its flexibility, or whether it represents a transitional state towards full insect or wind pollination. Wragg & Johnson found low levels of wind pollination in their putatively insect-pollinated species, and suggested that the presence of pollenkitt might limit the amount of pollen transfer by wind. The presence of pollenkitt facilitates pollen clumping and adherence to the bodies of generalist pollen-collecting insects. Thus, pollenkitt might be a key trait in ambophilous species for mediating the amount of pollen transferred by insects versus wind.

Although the transition from wind to animal pollination in the angiosperms is exceedingly rare, large-scale phylogenetic studies indicate that animal pollination is derived from wind pollination in families such as the Moraceae (Datwyler & Weiblen, 2004), Fagaceae (Manos *et al.*, 2001), and Salicaceae (Peeters & Totland, 1999). In all these families, the derived animal-pollinated group is an ancient and diverse lineage. This contrasts with the study by Wragg & Johnson, where the animal-pollinated species occur at the very tips of the large wind-pollinated Cyperaceae phylogeny. This distinction facilitates the use of a comparative approach to help understand the functional significance of the morphological differences between the insect- and wind-pollinated species.

A broad phylogenetic context provides the backbone for studying evolutionary transitions, and allows us to identify the direction of transitions and the correlated evolution of various characters. However, experiments like the one conducted by Wragg & Johnson are necessary to provide the functional basis for how transitions might occur and which characters are involved. To fully understand the selective mechanisms involved in the transition between animal and wind pollination (or vice versa), we need to incorporate experimental manipulations and common garden studies. Ideally, one would also want to identify the genes associated with the morphological changes. This could be achieved using a quantitative trait locus (QTL) approach by crossing closely related species (or ecotypes) with different pollination systems if these could be found. These approaches would provide a clearer picture of the causes and consequences of evolutionary transitions between animal and wind pollination.

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Key words: comparative analysis, evolutionary transition, flower color, insect pollination, wind pollination.