Natural History Note

Multifunctional Bracts in the Dove Tree *Davidia involucrata* (Nyssaceae: Cornales): Rain Protection and Pollinator Attraction

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abstract: Although there has been much experimental work on floral traits that are under selection from mutualists and antagonists, selection by abiotic environmental factors on flowers has been largely ignored. Here we test whether pollen susceptibility to rain damage could have played a role in the evolution of the reproductive architecture of *Davidia involucrata*, an endemic in the mountains of western China. Flowers in this tree species lack a perianth and are arranged in capitula surrounded by large (up to 10 cm × 5 cm) bracts that at anthesis turn from green to white, losing their photosynthetic capability. Flowers are nectarless, and pollen grains are presented on the recurved anther walls for 5–7 days. Flower visitors, and likely pollinators, were mainly pollen-collecting bees from the genera *Apis*, *Xylocopa*, *Halictus*, and *Lasioglossum*. Capitula with natural or white paper bracts attracted significantly more bees per hour than capitula that had their bracts removed or replaced by green paper. Experimental immersion of pollen grains in water resulted in rapid loss of viability, and capitula with bracts lost less pollen to rain than did capitula that had their bracts removed, suggesting that the bracts protect the pollen from rain damage as well as attracting pollinators.

Keywords: abiotic selection, *Davidia involucrata*, inflorescence architecture, pollinator attraction, pollen viability, pollen protection.

It has long been clear that angiosperm flowers can be fully understood only when considered as functional units,

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shaped by partly opposing selective pressures (Faegri and van der Pijl 1979; Waser and Ollerton 2006). Selection from mutualistic pollinators and antagonistic herbivores has received most attention (Grant and Grant 1965; Stebbins 1970; Johnson and Steiner 2000; Fenster et al. 2004; Strauss and Whittall 2006), but flowers are also under strong selection from their physical environment (Corbet 1990; Herrera 1993; Galen 2005). Among the abiotic selective agents on flowers is rain. This was first recognized by Sprengel ([1793]1972), who noted that rain may wash away pollen grains and dilute nectar. Later studies focused on losses in pollen viability resulting from prolonged immersion in water (Jones 1967; Eisikowitch and Woodell 1975; Corbet 1990; Huang et al. 2002). While some species have water-repellent pollen, others rely on nodding flowers, close their flowers by petal movements (Bynum and Smith 2001; Hase et al. 2006; He et al. 2006), or have other features protecting pollen from wetting. Thus, in species of the aquatic *Callitriche*, which are self-fertile, pollen tubes avoid contact with water by growing internally through vegetative tissues as they pass from male to female flowers, and in other aquatics, dry reproductive organs are maintained by modified flowers that close and entrap an air bubble when pulled below the water surface (Philbrick and Les 1996). In the erect flowers of *Primula vulgaris*, which regularly fill with water, roughly a quarter of the pollen grains still germinate even after 5 h underwater (Eisikowitch and Woodell 1975).

Experimental studies of the effects of rain on floral traits are surprisingly few (Bynum and Smith 2001; Galen 2005), and a recent review of nonpollinator selection on floral traits (Strauss and Whittall 2006) makes no mention of rain. Here we test whether the need to protect pollen grains from rain may have played a role in the evolution of the large bracts that surround the inflorescences of many Cornales, the dogwood clade.

Davidia involucrata Baillon, the only species in the genus, is a medium-sized tree (attaining heights of up to 20 m) that is endemic in mountain forests in western China (Fang and Chang 1983). Fossils from the Paleocene of

North America indicate that the lineage was more widespread in the past (for a review, see Manchester 2003). The English name, dove tree or handkerchief tree, refers to the two white, paperlike bracts that at anthesis surround the base of each flower head (capitulum). The bracts initially are small and green, resembling leaves, but increase in size and turn white as the flowers mature (fig. 1). The change in bract color from green to white is associated with the bracts becoming UV absorbing (Burr and Barthlott 1993). The anthers are even more strongly UV absorbing, and the species has therefore been classified as entomophilous (Burr and Barthlott 1993), although the pollination of *Davidia* had not been studied in the field. We carried out observations and experiments in a natural population in western China to assess the function of the bracts.

Methods

Study Species and Population

Davidia involucrata is native to western China, where it occurs in woodlands at altitudes of 1,100–2,600 m. Its dark purple inflorescences generally consist of a perfect flower surrounded by numerous male flowers (Fang and Chang 1983). The flowers are naked and nectarless, without sepals or petals. Before flowering, the bracts are green (fig. 1*a*), turning white rapidly as capitula mature and anthers begin to dehisce (fig. 1*b*). The anthers split longitudinally, and pollen grains are exposed on the recurved anther walls. Individual capitula of the dove tree last 5–7 days. The bracts drop off when anthesis ends. We manipulated flowers in a population of 16 flowering trees at Shennongjia
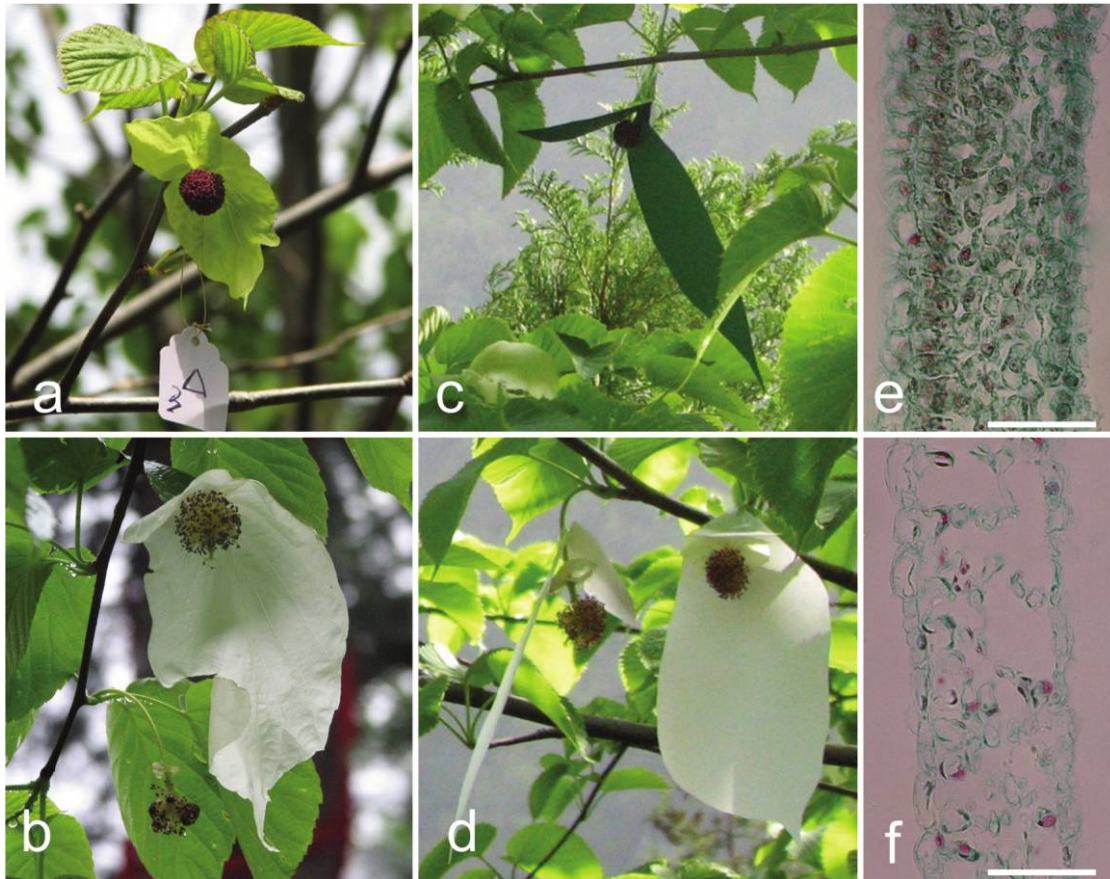

Figure 1: Manipulations of *Davidia involucrata* bracts to examine their role in pollinator attraction. *a*, Young inflorescence with green bracts and dark purple flower head; the anthers are immature. *b*, Mature inflorescences, the above one unmanipulated, the lower with both bracts removed. *c*, An artificial inflorescence in which white bracts were replaced by green paper. *d*, Two artificial inflorescences in which white bracts were replaced by white paper. *e*, Cross section of a green bract. *f*, Cross section of a white bract. Note the degeneration of mesophyll cells and chloroplasts in the white bract. Scale bars = 0.5 mm.
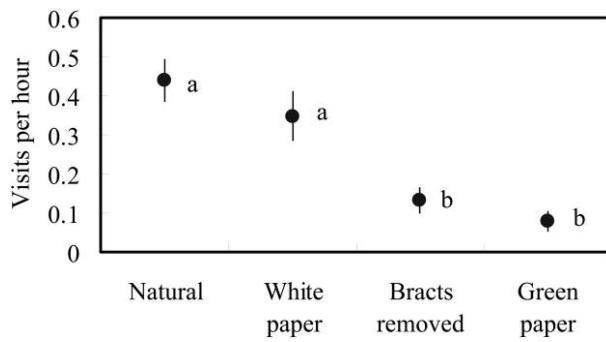
Figure 2: Numbers of bee visits per hour (mean ± SE, $n = 20$) to capitula ($n = 234$) subjected to four experimental treatments.

Nature Reserve, Hubei Province, in April 2005 and April 2006.

Bract Anatomy and Ontogeny

Green and white bracts were fixed in formalin–acetic acid–alcohol and after dehydration embedded in paraffin. Sections (8 μm) were stained with safranin and fast green. Between April 16 and April 22, 2006, the length and width of the bracts on each of 20 capitula were measured daily.

Pollination and Bract Function

Pollinator visits were recorded from 1000 hours to 1500 hours between April 18 and April 22 in 2005 and between April 16 and April 20 in 2006. Two observers monitored two sites during these periods of maximum pollinator activity. Insect visits became extremely rare after 1500 hours, and during one night of observations, no pollinators were observed visiting this nectarless species. Pollinators were collected and sent to the Institute of Zoology of the Chinese Academy of Science in Beijing for identification.

To investigate the role of bracts in pollinator attraction, we recorded pollinator visits to four kinds of capitula: (1) natural, (2) both bracts removed, (3) both bracts replaced by green artificial bracts made of copy paper (fig. 1*c*), or (4) both bracts replaced by white artificial bracts made of copy paper (fig. 1*d*). The artificial bracts were similar in shape and size to the natural bracts. To examine whether capitula with bracts lost less or more pollen to rain than did capitula that had their bracts removed, we counted pollen remaining within 29 and 27 capitula in late anthesis that were subjected to treatments 1 and 2, respectively, and had experienced at least one rainy day.

Numbers of anthers and ovules per capitulum and pollen grains produced per anther were estimated in 34 capitula with freshly dehiscing anthers. In September 2005

and 2006, we collected 39 and 32 open-pollinated capitula and counted their seeds to estimate seed set under natural pollination.

Pollen Stickiness and Behavior in Water

To assess the possibility of wind pollination, we tested pollen grain stickiness by their adherence to glass slides and examined seed production in 20 netted capitula. To test the behavior of pollen grains under rainy conditions, we followed Huang et al.'s (2002) method of pollen germination. Pollen grains from newly dehisced anthers were placed in sucrose solutions of 5%, 10%, 15%, and 20% by mass to examine optimum conditions for pollen germination. We then compared germination rates of pollen grains from eight randomly collected capitula in distilled water or in the optimum sucrose solution (10%). Grains that after 4 h had germinated or burst were counted under a light microscope. Ten samples of pollen grains from each flower were analyzed in this manner.

Data Analysis

One-way ANOVA analysis was used to compare the frequencies of pollinator visits to the four kinds of capitula and the amount of pollen remaining in capitula after different experimental treatments and to assess pollen viability in distilled water versus 10% sucrose solution.

Results

The green bracts' parenchyma cells were full of chloroplasts, while the parenchyma cells of white bracts had degenerated and contained few chloroplasts (fig. 1*e*, 1*f*). Bract color turned from green to white on the third or fourth day, when the anthers began to dehisce and when bracts had reached three-quarters of their final size (10.3 ± 0.3 cm × 4.6 ± 0.3 cm for the large bract and 4.5 ± 1.4 cm × 3.4 ± 0.9 cm for the small bract). Bracts continued to grow during anthesis but dropped off soon thereafter.

Pollen-collecting bees and pollen-feeding beetles were the major visitors and pollinators of the flowers. Wind pollination is unlikely to play a role because pollen grains are sticky and apparently not picked up by wind, nor did netted capitula produce any seeds. We recorded a total of 2,174 visits to capitula, of which bees and beetles accounted for 93.6% (2,034 visits) and 6.4% (140 visits), respectively. Beetles generally stayed within a capitulum for 1–2 h, while bees spent 4–6 s per capitulum foraging for pollen, suggesting that bees are the more effective pollinators of *Davidia involucrata*. Bees included *Apis cerana* Fabr., *Xylocopa appendiculata* Smith (Apidae), and *Halictus*
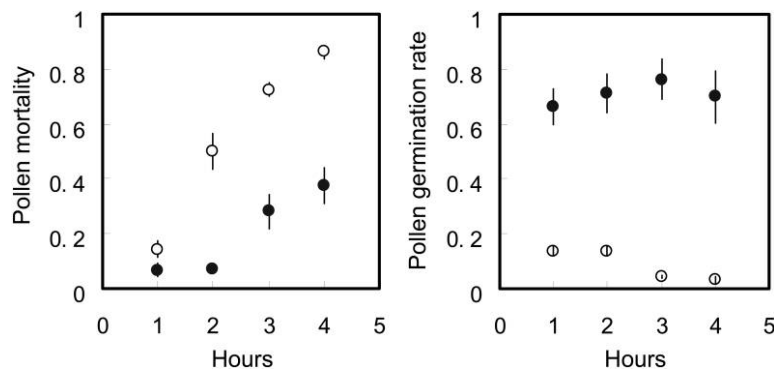
Figure 3: Percentage of pollen grains (mean ± SE) that burst or germinated following immersion in distilled water (*open circles*) or 10% sucrose solution (*solid circles*) over the course of 4 h.

and *Lasioglossum* species (Halictidae), and beetles included *Agriotes* species (Elateridae), *Oxycetonia jucunda* (Faldermann; Cetoniidae), and species of Nitidulidae. Visitor frequencies were low (0.5 ± 0.1 and 0.4 ± 0.1 visits per capitulum per hour for 48 and 125 h of observation in 2005 and 2006, respectively), and so was seed set of open-pollinated capitula (11.7% ± 2.3% and 39.7% ± 4.5%) in both years.

Bees preferred to visit capitula with white bracts over those with green bracts, on the basis of four consecutive days of observations on 62, 62, 62, and 48 inflorescences of natural capitula, bractless capitula, white-papered capitula, and green-papered capitula (fig. 2). Visits to natural (treatment 1) and white-papered (treatment 4) inflorescences were not significantly different ($F = 1.2$, df $= 1, 122$, $P = .27$) but were higher than those in the other two treatments ($F = 12.8$, df $= 3, 230$, $P < .0001$). Treatments 2 (bracts removed) and 3 (bracts replaced by green paper) did not differ significantly ($F = 1.6$, df $= 1, 108$, $P = .21$).

The pollen/ovule ratio in *D. involucrata* is extremely high, with a capitulum producing 6.6 ± 0.1 ovules and 954.4 ± 60.6 anthers with a total of 1,166,600 ± 96,900 pollen grains. Pollen amounts remaining in capitula with their bracts removed (186,650 ± 29,880) were not significantly different from those in intact capitula (148,000 ± 17,300) but were significantly lower than the total pollen production ($F = 80.8$, df $= 2, 87$, $P < .0001$), demonstrating high pollen loss to rain or removal by pollinators.

Pollen germinated best in 10% sucrose solution, and there was a highly significant difference in germination and bursting rates between pollen grains placed in distilled water or 10% sucrose solution (fig. 3). After 4 h, 86.3% ± 2.5% of pollen grains in water had burst, while only 37.5% ± 6.7% of the grains in 10% sucrose solution had burst ($n = 8$; $F = 46.9$, df $= 1, 14$, $P < .0001$).

Discussion

Pollen-collecting bees, the most important pollinators of *Davidia involucrata*, preferred visiting white-bracted capitula, and it is therefore likely that during their green stage, the bracts function in photosynthesis, while during their white UV-absorbing stage, they attract pollinators. During more than 170 h of observation, we never observed bees visiting green-bracted capitula. Their UV absorbance makes the white bracts stand out from surrounding foliage in the bee visual spectrum (Burr and Barthlott 1993; Kevan et al. 1996) and is likely due to the flavonoids that are the major pigments in the bracts of *D. involucrata* (Hu et al. 2007).

Over the 2 years of our study, insect visitation was low. With low visitation rates, prolonging anthesis will benefit reproductive success as a sit-and-wait strategy (Ashman and Schoen 1994). Protection of the pollen grains presented on the recurved anther walls during the 5–7 days of anthesis would then be of key importance and cannot be achieved other than by the bracts, since the flowers lack a corolla. Dove trees generally flower from mid-April to mid-May, that is, during the rainy season of the subtropical region where they occur. Given that in our experiments, pollen grains of *D. involucrata* burst in water (fig. 3), it is probable that the rooflike bracts function as an umbrella to reduce rain damage to the anthers and pollen. Indeed, we repeatedly observed rain-damaged androecia in bract-removed capitula (treatment 2), while bract-bearing capitula had dry stamens even after heavy rain (fig. 1*b*). That bract-bearing capitula "lost" as much pollen as bractless

ones was due to bees removing most pollen from the former, while rain washed away most pollen from the latter.

Flower or inflorescence structures with multiple functions may reflect the net effect of conflicting or additive selective pressures (Anderson 1976; Armbruster 1996, 1997, 2001; Galen 1999; Fenster et al. 2004; Armbruster et al. 2005; Strauss and Whittall 2006; Waser and Ollerton 2006). In the case of the living fossil *D. involucrata*, pollinator selection favors white bracts, while the need to protect pollen from rain favors large bracts. Notably, the two bracts differ in size (fig. 1), perhaps in response to constraints on weight or resources. Our observation of multifunctional bracts in the dove tree is mirrored in *Dalechampia* vines, in which bracts function as honest signals for the presence of floral reward (Armbruster et al. 2005) as well as in protection from florivores and pollen thieves (Armbruster 1997). Also in *Tilia*, the bracts not only help wind dispersal of the fruits but also may act as flags that attract pollinators, especially nocturnal pollinators (Anderson 1976). The bracts of the dove tree are a new striking example of transference of function from reproductive organs to vegetative organs (bracts being leaves) and of the multiple roles of one and the same structure in photosynthesis, pollinator attraction, and as a stamen rain shelter.

Acknowledgments

Literature Cited

Anderson, G. J. 1976. The pollination biology of *Tilia*. American Journal of Botany 63:1203–1212.

Armbruster, W. S. 1996. Evolution of floral morphology and function: an integrative approach to adaptation, constraint, and compromise in *Dalechampia* (Euphorbiaceae). Pages 241–272 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology. Chapman & Hall, New York.

———. 1997. Exaptations link the evolution of plant-herbivore and plant-pollinator interactions: a phylogenetic inquiry. Ecology 78:1661–1674.

———. 2001. Evolution of floral form: electrostatic forces, pollination, and adaptive compromise. New Phytologist 152:181–183.

Armbruster, W. S., L. Antonsen, and C. Pélabon. 2005. Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. Ecology 86:3323–3333.

Ashman, T. L., and D. J. Schoen. 1994. How long should flowers live? Nature 371:788–791.

Burr, B., and W. Barthlott. 1993. Untersuchungen zur Ultraviolettreflexion von Angiospermenblüten. II. Magnoliidae, Ranunculidae, Hamamelididae, Caryophyllidae, Rosidae. Tropische und subtropische Pflanzenwelt 87, Akademie der Wissenschaften und der Literatur, Mainz Franz Steiner, Stuttgart.

Bynum, M. R., and W. K. Smith. 2001. Floral movements in response to thunderstorms improve reproductive effort in the alpine species *Gentiana algida* (Gentianaceae). American Journal of Botany 88:1088–1095.

Corbet, S. A. 1990. Pollination and the weather. Israel Journal of Botany 39:13–30.

Eisikowitch, D., and S. R. J. Woodell. 1975. The effect of water on pollen germination in two species of *Primula*. Evolution 28:692–694.

Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology. 3rd ed. Pergamon, Oxford.

Fang, W.-P., and C.-Y. Chang. 1983. Flora Republicae Popularis Sinicae. Vol. 52. Science, Beijing.

Fenster, C. B., W. S. Armbruster, P. Wilson, R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology Evolution and Systematics 35:375–403.

Galen, C. 1999. Why do flowers vary? the functional ecology of variation in flower size and form within natural plant populations. BioScience 49:631–640.

———. 2005. It never rains but then it pours: the diverse effects of water on flower integrity and function. Pages 77–95 *in* R. Edward and F. A. Bazzaz, eds. Reproductive allocation in plants. Elsevier Academic, Burlington, MA.

Grant, V., and K. A. Grant. 1965. Flower pollination in the phlox family. Columbia University Press, New York.

Hase, A. V., R. M. Cowling, and A. G. Ellis. 2006. Petal movement in cape wildflowers protects pollen from exposure to moisture. Plant Ecology 184:5–87.

He, Y.-P., Y.-W. Duan, J.-Q. Liu, and W. K. Smith. 2006. Floral closure in response to temperature and pollination in *Gentiana straminea* Maxim. (Gentianaceae), an alpine perennial in the Qinghai-Tibetan Plateau. Plant Systematics and Evolution 256:17–33.

Herrera, C. M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. Ecological Monographs 63:251–275.

Hu, J.-Y., S.-L. Zhang, Z.-X. Su, and Y.-M. Liao. 2007. Pollinator attraction by *Davidia involucrata*. I. Color. Journal of Plant Ecology 31:166–171. [In Chinese with English abstract.]

Huang, S.-Q., Y. Takahashi, and A. Dafni. 2002. Why does the flower stalk of *Pulsatilla cernua* (Ranunculaceae) bend during anthesis? American Journal of Botany 89:1599–1603.

Johnson, S. D., and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. Trends in Ecology & Evolution 15:140–143.

Jones, C. E. 1967. Some evolutionary aspects of a water stress on flowering in the tropics. Turrialba 17:188–190.

Kevan, P., M. Giurfa, and L. Chittka. 1996. Why are there so many and so few white flowers? Trends in Plant Science 1:280–284.

Manchester, S. R. 2003. Leaves and fruits of *Davidia* (Cornales) from the Paleocene of North America. Systematic Botany 27:368–382.

Philbrick, C. T., and D. H. Les. 1996. Evolution of aquatic angiosperm reproductive systems. BioScience 46:813–826.

Sprengel, C. K. (1793) 1972. Das entdeckte Geheimnis der Natur im

Bau und in der Befruchtung der Blumen. Weldon & Wesley, New York. Original edition, Vieweg, Berlin.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. Annual Review of Ecology and Systematics 1:307–326.

Strauss, S. Y., and J. B. Whittall. 2006. Non-pollinator agents of selection on floral traits. Pages 120–138 *in* L. D. Harder and S. C.

H. Barrett, eds. Ecology and evolution of flowers. Oxford University Press, Oxford.

Waser, N. M., and J. Ollerton. 2006. Plant-pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago.