MORPHOLOGICAL STUDIES OF THE NYMPHAEACEAE SENSU LATO. XVI. THE FLORAL BIOLOGY OF *BRASENIA SCHREBERI*[1]

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ABSTRACT

Observations conducted in East Texas on the pollination biology of Brasenia schreberi *J. F. Gmel. confirm that anthesis is diurnal, with individual flowers opening and closing for two consecutive days. First-day flowers are morphologically and functionally pistillate. They are characterized by short, undehisced stamens and elongated, papillate stigmas that radiate outward over the perianths, providing an expanded surface area for pollen adherence. Second-day flowers are morphologically and functionally staminate. Staminal filaments are elongated, elevating the dehiscent anthers to a position above the centrally aggregated stigmas. Although self-pollination occurs, dichogamy prevents individual flowers from self-fertilizing.* Notiphila cf. cressoni *(Diptera; Ephydridae) was the most frequent insect visitor. Based on behavior and pollen loads, insect pollination is insignificant. The expanded stigmatic surface area, exserted stamens, and additional floral and vegetative features are adaptations for wind pollination. Data from pollen dispersal experiments indicate that anemophily is the primary mechanism of pollen transfer. This pollination mechanism is unique in the* Nymphaeaceae sensu lato. *Evidence from pollination biology, floral anatomy, seed anatomy, and embryology indicates a close evolutionary relationship between the Cabombaceae and Nymphaeaceae sensu stricto. Genera of the* Nymphaeaceae s. str. *and Nelumbonaceae exhibit a phyletic elaboration of the flower, whereas the Cabombaceae represent a phyletic reduction.*

Brasenia schreberi J. F. Gmelin is a monotypic genus with a wide but sporadic distribution in lakes, ponds, and slow streams. It occurs in eastern Asia, Australia, Africa, the West Indies, and South, Central, and North America. In North America the species ranges from Florida to East Texas, north to Prince Edward Island, southern Quebec, southern Ontario, and Minnesota. It also occurs in Idaho, California, north to British Columbia, and Alaska (Wood, 1959). Although *Brasenia* does not presently occur in Europe, fossil specimens are known (Srodon, 1935; Tralau, 1959; Jessen et al., 1959; Hall, 1978; Collinson, 1980). *Brasenia* is commonly known as water-shield, water-target, purple bonnet, and purple wen-dock and is a cultivated food source in Japan (Matsuda & Hara, 1985).

Brasenia is one of eight genera within the Nymphaeaceae sensu lato as circumscribed by Bentham & Hooker (1862). Subsequent workers have grouped the nymphaeaceous genera into various orders, families, and tribes (for review see Goleniewska-Furmanowa, 1970; Takhtajan, 1980; Cronquist, 1981). The opinions that the genera should be divided among three subfamilies or three families (Nymphaeaceae, Nelumbonaceae, and Cabombaceae) within the order Nymphaeales has been widely advocated on the basis of available data. A ninth genus, *Ondinea*, was described by den Hartog (1970) and placed within the Nymphaeaceae sensu stricto. This taxonomic alignment is supported based by studies of seed anatomy, floral morphology, and floral biology (Schneider, 1978, 1983).

In all systematic treatments, *Brasenia* has been allied with *Cabomba* on the basis of such shared morphological features as long, slender, sympodial stems; peltate floating leaves; small hypogynous flowers with apocarpous gynoecia, and few floral parts. Moseley et al. (1984), comparing anatomical

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and morphological features of the vegetative axes and flowers in *Cabomba* and *Brasenia*, suggested that the two genera are more distant taxonomically than envisioned by earlier workers (Vinogradov, 1967; Goleniewska-Furmanowa, 1970; Bukowiecki et al., 1972, 1974), a view also shared by Collinson (1980). Moseley et al. emphasized, however, that the taxonomic distance was not adequate to warrant dismantling the Cabombaceae. Richardson (1969) concluded that the floral vascular system (and by inference, the flower) of *Brasenia* exhibits a phylesis toward condensation and reduction from a more primitive, larger-flowered ranalian ancestor. Ito (1986a) determined that the ontogeny and anatomical construction of the receptacular vascular plexus, a feature common to all water lily genera, differs in the Cabombaceae from those found in the Nymphaeaceae s. str. Ito also considered the floral vasculature of *Cabomba* to be derived (via reduction in the number of stamens and carpels) from the type of vasculature found in the flower of *Brasenia*.

Numerous studies of *Brasenia* involving a diversity of features have been conducted including: seed anatomy and embryology (Weberbauer, 1893, 1894; Chifflot, 1902; Cook, 1906, 1909; Melikyan, 1964; Khanna, 1965; Corner, 1976); pollen morphology (Wodehouse, 1932; Ikuse, 1955; Ueno & Kitaguchi, 1961; Ueno, 1962; Meier, 1964; Walker & Doyle, 1975; Walker, 1976a, b; Clark & Jones, 1981; Batygina & Shamrov, 1983); xylem anatomy (Kosakai, 1968); karyology (Langlet & Söderberg, 1927; Okada & Tamura, 1981); chemical analyses (Nakahara, 1940; Riemer & Toth, 1970; Goleniewska-Furmanowa, 1970; Kakuta & Misaki, 1979; Sevilla et al., 1984); leaf development and anatomy (Goleniewska-Furmanowa, 1970; Kaul, 1976; Chen & Zhang, 1986); floral anatomy (Troll, 1933; Moseley, 1958; Khanna, 1965; Richardson, 1969; Moseley et al., 1984; Ito, 1986a); paleobotany (Hall, 1978; Collinson, 1980; Dorofeev, 1984); and general morphology and taxonomy (Lawson, 1888; Schrenk, 1888; Caspary, 1891; Keller, 1893; Raciborski, 1894; Schilling, 1894; Gwynne-Vaughn, 1897; Hill, 1900; Chrysler, 1938; Wood, 1959; Adams, 1969; Kristen, 1974; Ogden, 1974; Rao & Banerjee, 1979; Matsuda & Hara, 1985). Little attention, however, has been given to the floral (pollination) biology of the genus. Tokura (1937) investigated the blooming of *Brasenia* in Japan and was the first to record the movements of floral parts during the two-day anthesis period. Tokura suggested that flowers on the first day of anthesis are pistillate, while on the second day staminate, during which

time large quantities of pollen are released. Tokura noted the importance of the increased height of second-day, pollen-releasing flowers above those of the first-day, pollen-receptive flowers from the standpoint of pollen transfer but did not suggest the vector(s) for pollination. Schneider & Jeter (1982) reported "*Notiphila*-like" flies functioning as pollinators in populations of *Brasenia* growing in East Texas.

This investigation is part of a continuing series of studies designed to contribute new evidence for determining relationships among water lily genera. It is the objective of this study to: (1) confirm and amplify observations on floral morphology and floral behavior during anthesis of *Brasenia*; (2) elucidate the mechanism(s) of pollen transfer and relate floral morphology to pollination syndrome(s); and (3) compare the pollination biology of *Brasenia* with other genera of Nymphaeaceae s.l. This may contribute to a better understanding of the phylogeny of this angiosperm family, which occupies a basal, pivotal systematic position in many old and modern classification schemes.

MATERIALS AND METHODS

SITE DESCRIPTION

Observations on the floral biology of *Brasenia* were conducted during the summers of 1986 and 1987 in Toledo Bend Reservoir, Sabine County, Texas. Extensive populations of *Brasenia* exist in numerous coves throughout the reservoir. This study was conducted in a cove of six surface hectares adjacent to the Willow Oak Recreation Area. The water level within the reservoir fluctuated widely throughout the study period. The depth of water in which *Brasenia* grew ranged from 0.5 to 2.5 m.

FLORAL CYCLE

Ten flowers in various stages of anthesis were tagged with numbered fluorescent orange corks by securing them to peduncles with nylon fishing line. Heights of the floral structure above and below water level were measured over four consecutive days. Measurements were made from the base of the receptacle to the water level. In addition, sepal, petal, staminal, and stigmatal positions were observed.

CAGING TREATMENTS

Exclusion treatments were accomplished by placing floating cages over emergent flowers for the duration of anthesis. Flowers were tagged with fluorescent corks attached to the peduncle for fruit

retrieval. Different experiments were identified by color-coded survey ribbons stapled to corks. Cages consisted of 950-ml styrofoam and plastic drinking cups mounted inversely on 21-cm² styrofoam sheets. The center portion of the styrofoam base was removed to allow placement of cages over flowers. Two types of cages were used: one to exclude all abiotic and biotic pollen vectors, and a second to exclude only biotic vectors. Abiotic and biotic exclusion cages were made from clear plastic cups (transparent cages). Biotic exclusion cages were produced using styrofoam cups with four rectangular windows cut out around their circumferences. Windows were covered with 1-mm² fiberglass mesh screen glued to the styrofoam cups (mesh cages). Transparent cups were mounted on 2.5-cm-thick styrofoam, while mesh cups were mounted on 1.3-cm-thick styrofoam sheeting to maximize flower exposure through the mesh windows above the styrofoam base. Both types of cups were mounted to the styrofoam bases with water-insoluble glue.

Three control groups, each consisting of 25 second-day flowers, were tagged during intervals throughout the study period to determine natural seed set. An additional control group, consisting of 25 flowers that had morphologically short stigmas, was also tagged. Each exclusion treatment involved various floral manipulations of 25 first-day flowers and their subsequent seed production to check for the following:

Parthenocarpy (Group A). Flowers were covered prior to anthesis with transparent cages and emasculated. Emasculation involved the removal of undehisced anthers on the first day of anthesis. Cages were observed periodically throughout anthesis to check flower position and condition.

Autogamy (Group B). Flowers were covered with transparent cages and left undisturbed.

Stigmatic receptivity (Group C). (1) Flowers were covered with transparent cages and emasculated. First-day stigmas were dusted copiously with pollen transferred mechanically from uncaged pollen-releasing flowers. (2) Flowers were covered with transparent cages and emasculated. Pollen was transferred mechanically to second-day stigmas.

Allogamy (Group D). (1) Geitonogamy. Flowers were covered with transparent cages and mechanically cross-pollinated on the first day with pollen from uncaged flowers from the same plant. (2) Xenogamy. Flowers were covered with transparent cages and cross-pollinated on the first day with pollen from uncaged flowers from different plants.

Anemophily (Group E). (1) Flowers were covered with mesh cages and left undisturbed. (2) Flowers were covered with mesh cages and emasculated.

POLLEN DISPERSAL AND TERMINAL SETTLING VELOCITY

The presence of wind-borne *Brasenia* pollen was determined using an Andersen 0101 particle-size air sampler (Andersen, 1958). In addition, dispersed pollen was quantified by measuring distance and angle of dehiscence from pollen-releasing flowers. Measurements were made using calibrated poster boards 71.5 × 56 cm. Black poster boards (pollinometers) were marked in increments of 10 cm and 20° from the midpoint of one edge (Fig. 1). Circles equal in size to an average stigmatic surface area of a first-day flower were drawn into distance-angle segments. Boards were laminated and mounted on 2.5-cm-thick styrofoam.

Individual pollinometers were placed downwind from single pollen-releasing flowers with the peduncle at the 0° mark between 0800–1200 hours. All additional pollen-releasing flowers from the surrounding 3-m radius were removed. Pollinometers were retrieved after total anther dehiscence, and the number of pollen grains within each circle was quantified using a hand lens. Samples were taken on two separate days, six trials per day. Wind-speed measurements were taken using a Taylor anemometer. Results were analyzed using Student's *t*-test and regression analyses.

Stigmas were viewed with a dissecting microscope to detect the presence of pollen. These included stigmas on uncaged first-day flowers in close proximity and downwind of pollen-releasing flowers and uncaged first-day flowers not adjacent to pollen-releasing flowers. Comparisons were made of pollen quantity on stigmas of flowers in each condition. Additionally, pollen distribution was observed (e.g., more grains on leeward or windward surfaces).

The terminal settling velocity of freshly collected *Brasenia* pollen was determined utilizing the stroboscopic photography techniques of Niklas (1984).

POLLEN—OVULE RATIOS AND POLLEN VIABILITY

The number of pollen grains/flower was determined by suspending all pollen from 10 anthers in a 0.5-ml solution of aniline-blue in lactophenol and counted using a hemacytometer (Cruden, 1977). Pollen—ovule ratios were calculated assuming a mean number of 28 anthers and 12 carpels (24
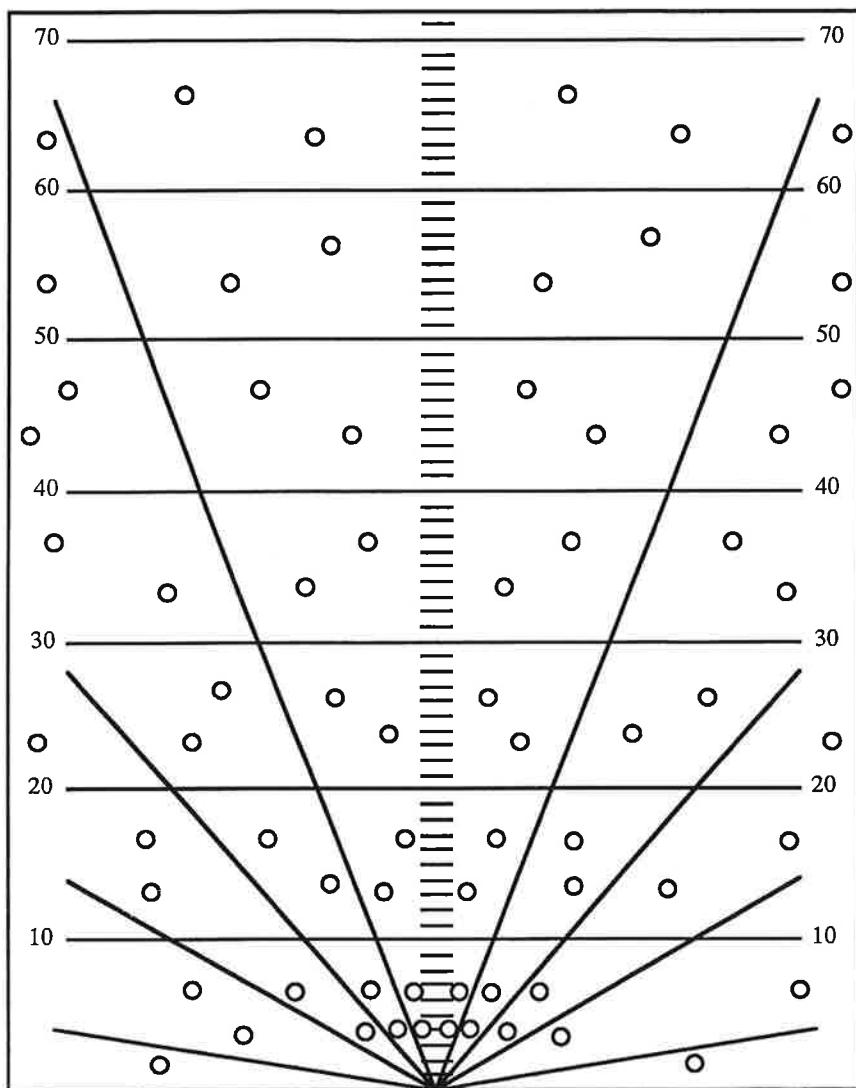
FIGURE 1. *Pollinometer. Circles represent the average stigmatic surface area of first-day flowers. Seventy cm scale.*

ovules) per flower. Pollen viability was determined using aniline-blue in lactophenol staining.

FLORAL DENSITY

The distributional density of flowers was determined by counting the total number of first- and second-day flowers within ⅛-m frames in a random stratified design.

FLORAL SECRETIONS

The presence of floral secretory tissues was determined using neutral red stain as an indicator (Esau, 1965; Vogel, 1966). Fresh first- and second-day flowers were placed in neutral red for three to six hours. After excess stain was removed by lightly washing with water, the flowers were examined using a dissecting microscope.

ULTRAVIOLET REFLECTANCE AND ABSORPTION

Ultraviolet (UV) photographs using black and white Kodak Plus X film, 125 ASA, and a Kodak Wratten ultraviolet filter No. 18A were made of first-day and second-day flowers and leaves in sunlight. Second-day flowers were photographed prior and subsequent to anther dehiscence.
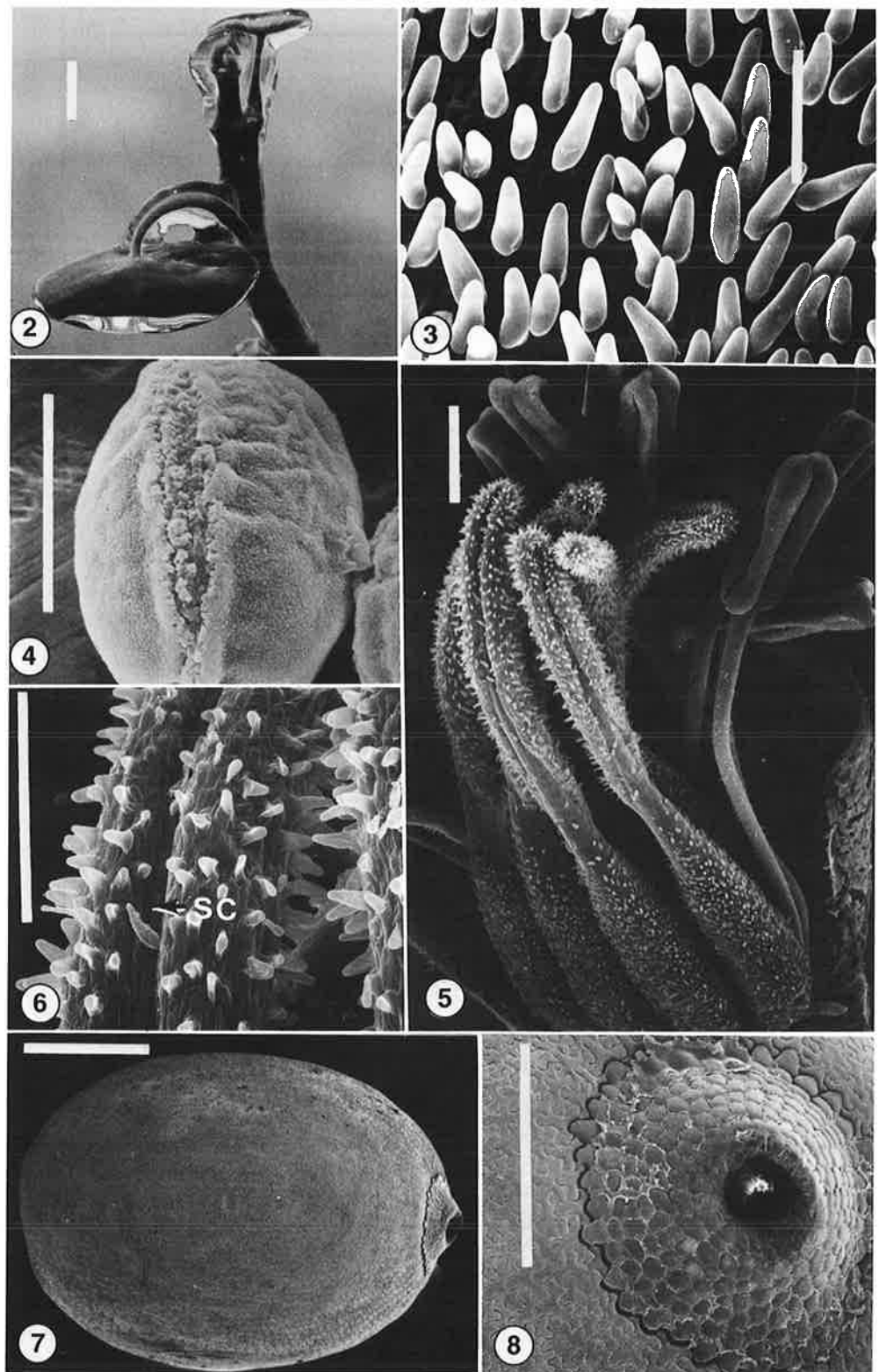
ANTHOPHILOUS VISITORS

Observations were made to determine diversity, frequency, behavior, and extent of pollen loads of various insect visitors. Insects on flowers and leaves were collected using kill jars with ethyl acetate and were preserved in 70% ethanol. Voucher specimens are housed at SWTSU.

SCANNING ELECTRON MICROSCOPY

Floral and fruit specimens were investigated with a Cambridge S90 scanning electron microscope (SEM). Tissues were fixed for 24 hours in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. Samples were washed with the buffer and postfixed in 1% osmium tetroxide for three hours, then stored in the buffer. Floral specimens were dehydrated in acidified dimethoxypropane (Postek & Tucker, 1976; Linn et al., 1977) and critical-point dried. Seeds were immersed in acetone and placed in an ultrasonicater for five minutes and air dried. Pollen grains were pipetted onto filter paper and air dried. Floral and seed specimens were sputter-coated with gold or gold–palladium and then mounted on aluminum stubs with colloidal graphite. Pollen grains were mounted by inverting finely polished stubs covered with Mikrostik® onto filter papers with the dried pollen and then sputter-coated.

OBSERVATIONS

HABIT

Brasenia is a rhizomatous, aquatic perennial. The rhizomes bear axillary buds, adventitious roots, and leaves at each node. Leaves are alternate, long-petioled, and centrally peltate. Young leaves are involute in bud. Mature leaf blades are floating and oval to elliptic with entire margins. Submerged stems, petioles, and abaxial leaf surfaces are heavily coated with a layer of transparent mucilage, as are young plant parts such as axillary buds and juvenile leaves (Fig. 2).

Flowers of *Brasenia* are about 2 cm in diameter, dull purple, and emergent. They are borne singly on long, mucilage-coated peduncles and possess linear-lanceolate perianth parts with three petaloid sepals and three petals. All perianth members bear antrorse, adaxial trichomes (Fig. 3).

The androecia of flowers within the study site are composed of 24–33 stamens with filiform filaments and four apical microsporangia. Dehiscence is latrorse in the study populations. Pollen grains are elliptic, monosulcate, and have faintly scabrate ornamentation (Fig. 4). The pollen–ovule ratio of *Brasenia* is $9,238 \pm 625$ (95% C.I., N = 11), and 98% of the pollen is viable.

Flowers are hypogynous with apocarpous gynoecia of 10–14 carpels in Toledo Bend populations. These are characterized by relatively short styles and by linear, extremely papillate stigmas with abaxial stigmatic crests (Figs. 5, 6). Ovaries contain one or two anatropous and crassinucellate ovules. Placentation ranges from laminar to dorsal (Richardson, 1969) to median (Ito, 1986a).

Fruits are aggregate and subtended by a persistent perianth. Each simple fruit is indehiscent, one- or two-seeded, and surrounded by a leathery pericarp. Peduncles bearing aggregate fruits abscise, float to the surface, and drift. Eventually, simple fruits detach from the receptacle, float on the water's surface where they are carried by wind and wave actions, and then sink to the pond bottom.

The seeds are ovoid (Fig. 7) and at their apex possess a pyramidal structure with a central micropyle (Fig. 8). The seed coat surface is composed of irregularly digitate cells. Seeds contain small amounts of endosperm, copious perisperm, and haustorial tubes. Embryos are minute, with two broad hemispherical cotyledons (Fig. 9).

FLORAL CYCLE

The floral structure of *Brasenia* is diurnally emergent over a three-day period. On the first day of emergence, the flower is in bud and covered with mucilage (Fig. 10). Individual flowers bloom for two consecutive days. On the first day, flowers are morphologically pistillate; they are characterized by short, undehisced stamens and elongate, papillate stigmas radiating outward over the re-

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FIGURES 2–8. *Morphological and anatomical features of* Brasenia.*—2. Axillary bud and juvenile leaves; note abundant mucilage covering entire apex and abaxial surface of older leaf. Scale bar = 1 cm.—3. SEM of adaxial surface of sepal; note numerous antrorse trichomes. Scale bar = 1 mm.—4. SEM of monosulcate pollen grain. Scale bar = 20 µm.—5. SEM of second-day flower showing carpel morphology; note linear stigmas with abundant papillae and stigmatic crests. Scale bar = 1 mm.—6. SEM of papillate stigma; note abaxial stigmatic crest (SC). Scale bar = 500 µm.—7. SEM of seed. Scale bar = 1 mm.—8. SEM of seed apex; note digitate cells of seed surface and polygonal cells of apex surface. Scale bar = 500 µm.*
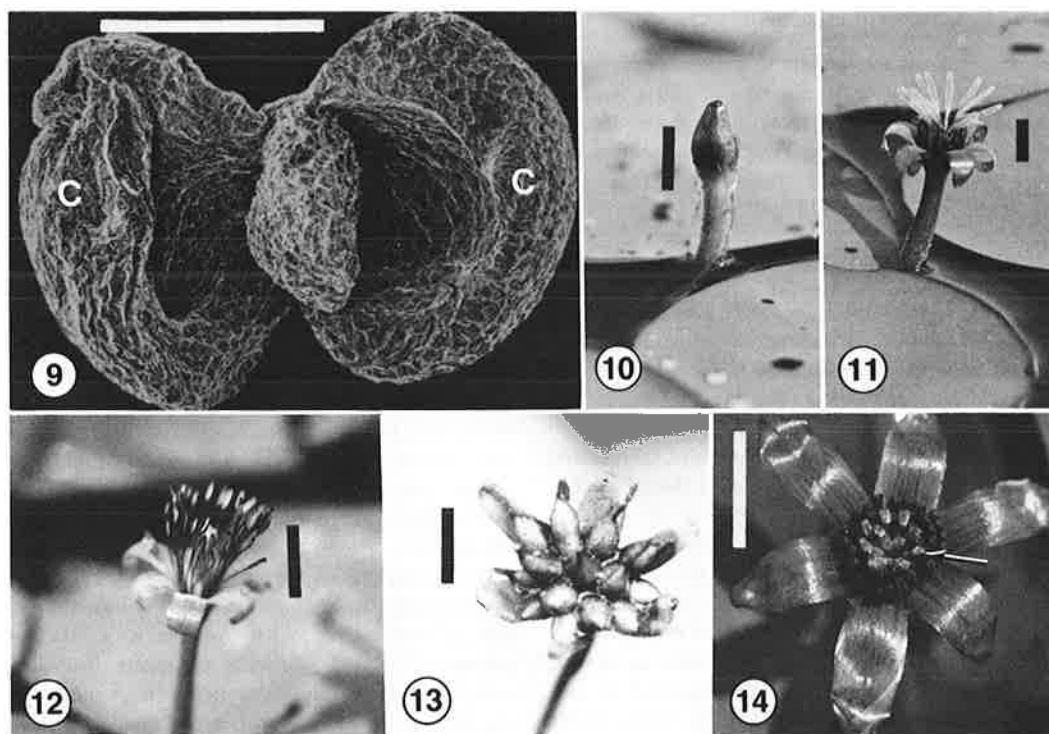
FIGURES 9–14. *Embryo and flowers of* Brasenia.—*9. SEM of embryo with hemispherical cotyledons (C). Scale bar = 250 μm.—10. Floral bud; note mucilage-covered peduncle.—11. First-day flower showing short, undehisced anthers and radiating stigmas.—12. Second-day flower showing elongated filaments, dehiscent anthers, and centrally aggregated stigmas.—13. Aggregate fruit.—14. First-day flower of* Brasenia *showing variation in lengths of stigmas. (Figures 10–14, scale bars = 1 cm.)*

flexed perianth (Fig. 11). Flowers submerge at the end of the first day. On the second day, the flowers are morphologically staminate; the filaments have elongated, elevating the dehiscent anthers to a position at or above the now centrally aggregated stigmas (Fig. 12). The perianth occupies a position similar to that of first-day flowers. At the end of the second day, flowers again submerge. Third-day flowers remain submerged but occasionally occur at the water surface. These flowers are nonfunctional, characterized by closed perianth parts with protruding senescent anthers. Aggregate fruits develop below the water surface (Fig. 13). Fruit development occurs in four to six weeks early in summer and as quickly as two weeks in the late summer.

During the course of fieldwork, morphological variation was observed among flowers. Aside from typical features described above, several *Brasenia* flowers had short stigmas which did not conspicuously radiate over the perianth (Fig. 14).

Individual flowers vary in position above and below the water level. General trends of the floral cycle can be identified, however (Fig. 15). Anthesis begins at 0630–0730 hours on both the first and second days. First- and second-day flowers reach maximum height above water at 1300–1400 hours. Second-day flowers are generally elevated to a higher position, with anther dehiscence occurring at 0830–1100 hours.

As first-day flowers close, the gynoecia and perianths gradually change position. When flowers are at peak height, stigmas begin to arch centrally until they completely aggregate at the time flowers close and submerge. Perianth members begin to close gradually at 1300–1400 hours, with the corolla closing prior to the calyx.

CAGING TREATMENTS

Results from caging experiments are reported as percentage seed set (Table 1). The percentage values are conservative because they were calculated assuming two ovules (seeds) per carpel (simple fruit) within carpels remaining on aggregate fruits at time of retrieval. They do not take into account
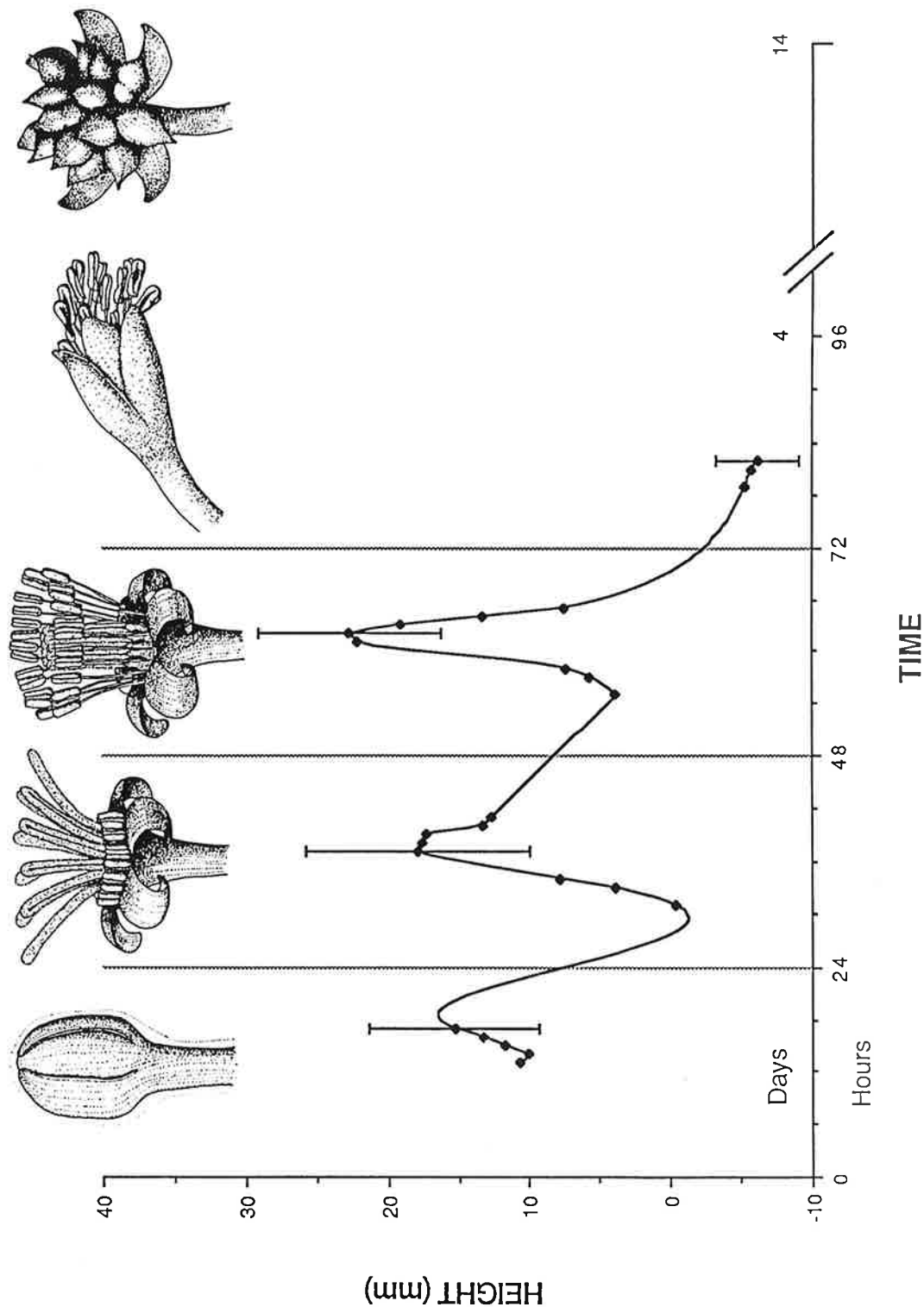
FIGURE 15. *Floral cycle of Brasenia illustrating changes in floral morphology and position above or below water level. Error bars represent average 95% confidence intervals of all values from each day; buds C.I. = 6.06, first-day flowers C.I. = 7.92, second-day flowers C.I. = 6.41, and third-day flowers C.I. = 2.83. Best-fit curve was computer generated.*

TABLE 1. *Percentage seed set from caging treatments conducted on separate trial dates.*

Experiment (N = 25)	5/23/86	6/19/86	7/03/86	7/31/86	5/18/87
Control					
1. Typical flowers	18.2%	6.1%	—	—	28.7%
2. Flowers with short stigmas	—	—	—	—	1.1%
Group A—Parthenocarpy					
(Transparent cages, emasculated)	—	0%	—	—	—
Group B—Autogamy[1]					
(Transparent cages, left undisturbed)	—	0%	—	—	—
Group C—Stigmatic receptivity					
1. Transparent cages, emasculated, cross-pollinated first-day	—	—	12.7%	—	—
2. Transparent cages, emasculated, cross-pollinated second-day	—	—	0.5%	—	—
Group D—Allogamy[1]					
1. Geitonogamy (transparent cages, cross-pollinated first-day with pollen from same plant)	—	—	—	—	48.0%
2. Xenogamy (transparent cages, cross-pollinated first-day with pollen from different plant)	—	—	—	—	41.4%
Group E—Anemophily					
1. Mesh cages, left undisturbed	—	0%	—	1%	—
2. Mesh cages, emasculated	—	—	0%	—	—

[1] Terminology follows Faegri & van der Pijl (1979).

any carpels that had abscised prior to retrieval or carpels with only one ovule.

The results of exclusion treatments indicate that apomixis and autogamy did not occur (Groups A and B). Self-pollination did occur in treatment B, however, based on the observation of pollen on the stigmas. Flowers artificially pollinated on the first day and second day of anthesis (Group C) produced seeds. Flowers pollinated on the first day of anthesis exhibited greater seed set than those pollinated on the second day. These data indicate first-day flowers are receptive to pollen and should be considered functionally pistillate. Second-day flowers are functionally staminate. The results of experiment D, determination of allogamy, revealed that *Brasenia* is compatible with pollen of both geitonogamous and xenogamous origins. Only one of three trials from the mesh cage treatments (Group E) set seed. It is believed that minimal seed set in flowers subjected to this treatment occurred because the cages alter the aerodynamics of the floral structure (Niklas, pers. comm.) and the mesh screen probably inhibits the passage of pollen grains.

The three control groups of morphologically "typical" flowers yielded varying seed set (Table 1). This variation may be attributed to differences in wind speed and distributional density of flowers on each sample date. Flowers with short stigmas produced few seeds despite their occurrence in the same population as the "typical" control group.

POLLEN DISPERSAL AND TERMINAL SETTLING VELOCITY

Measurements with the Andersen particle sampler revealed that *Brasenia* pollen was airborne. Pollinometer experiments indicated the mean number of pollen grains dispersed from second-day (pollen-releasing) flowers decreases with distance. The numbers of grains at subsequent 10 cm intervals on each trial date were significantly different, with the exceptions of the 0–10 and 60–70 cm intervals. These differences can be attributed to wind velocity. Average wind speeds on respective sampling dates differed by a factor of five. Dispersion of pollen can be described exponentially (Fig. 16). A regression analysis of dispersed pollen grains exhibited correlation coefficients of $r = 0.99$ and $r = 0.85$ at 8 kph and 1.6 kph, respectively.

Pollen on the stigmas of a variety of flowers was also observed. First-day flowers that were downwind of and within 0.5 m of second-day flowers had abundant pollen on their stigmas. Those grains were primarily restricted to windward stigmas. Pol-
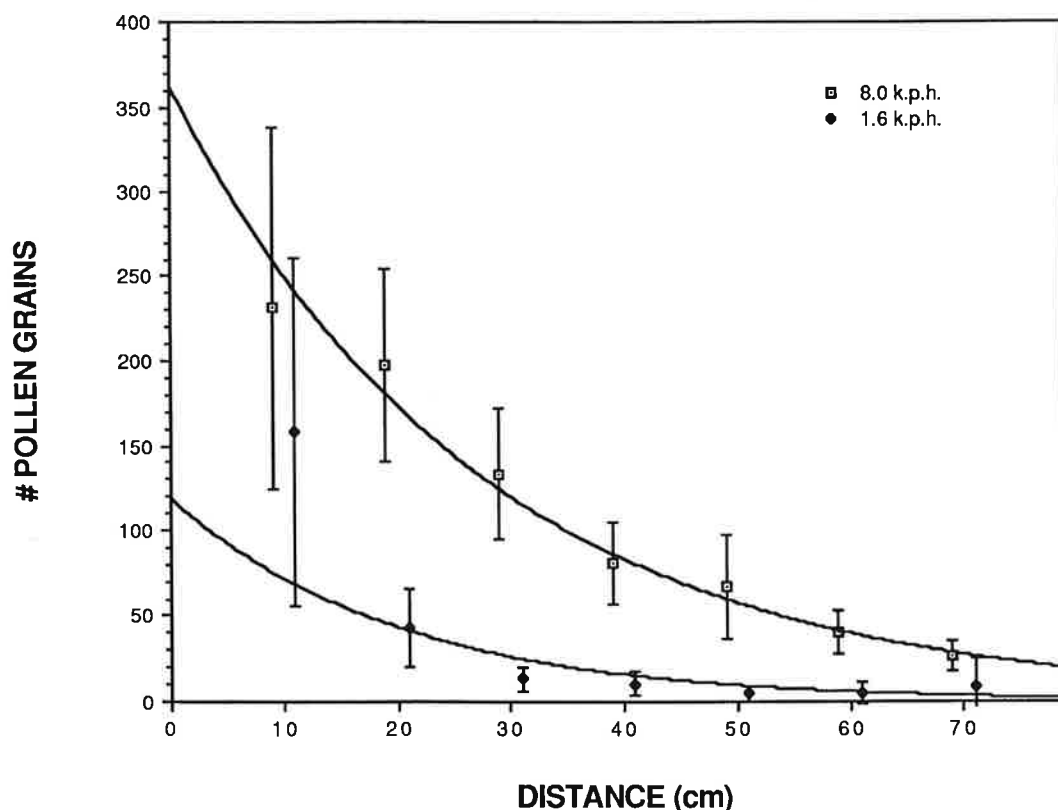
FIGURE 16. *Regression analysis of pollen grain deposition from pollinometer experiments. At 8 kph, y = 367.9*10^ (0.02x), r value of 0.99; and at 1.6 kph, y = 112.7*10^ (0.02x), r value of 0.85. Error bars represent 95% confidence intervals; best-fit curves were computer generated.*

len-receptive first-day flowers that were 2–3 m from second-day flowers exhibited very low quantities of pollen, if any, on their stigmas. The terminal settling velocity of *Brasenia* pollen was calculated to be 7.7 ± 0.8 (95% C.I., N = 21) cm/sec.

FLORAL DENSITY

Of four sample plots, the mean numbers of flowers/m^2 ± 95% C.I. were as follows: 110 ± 22; 82 ± 26; 73 ± 17; and 36 ± 14.

FLORAL SECRETIONS

Neutral red staining revealed the absence of nectaries. The numerous perianth trichomes stained indicated a secretory role.

ULTRAVIOLET REFLECTANCE AND ABSORPTION

The inner surfaces of dehisced anthers are UV reflective. Adaxial (emergent) leaf surfaces are UV absorptive.

ANTHOPHILOUS VISITORS

A variety of insects visit flowers of *Brasenia*, including *Donacia cincticornis* Newman (Coleoptera; Chrysomelidae), *Perigaster cretura* Herbst (Coleoptera; Curculionidae), *Notiphila* cf. *cressoni* Cresson (Diptera; Ephydridae), *Apis mellifera* L. (Hymenoptera; Apidae), and various odonates. The most frequent and abundant visitor was *Notiphila* cf. *cressoni*. *Notiphila* primarily visited staminate flowers and there foraged for pollen either directly from the dehisced anther sacs or from the adaxial surface of the reflexed perianth where pollen accumulates (Fig. 17). Grooming and copulatory acts were also frequently observed. Pistillate flowers were rarely visited by flies, but upon their brief visitations, members of *Notiphila* would typically land on the elongated stigmas or reflexed perianth (Fig. 18). Additionally, flies commonly foraged for
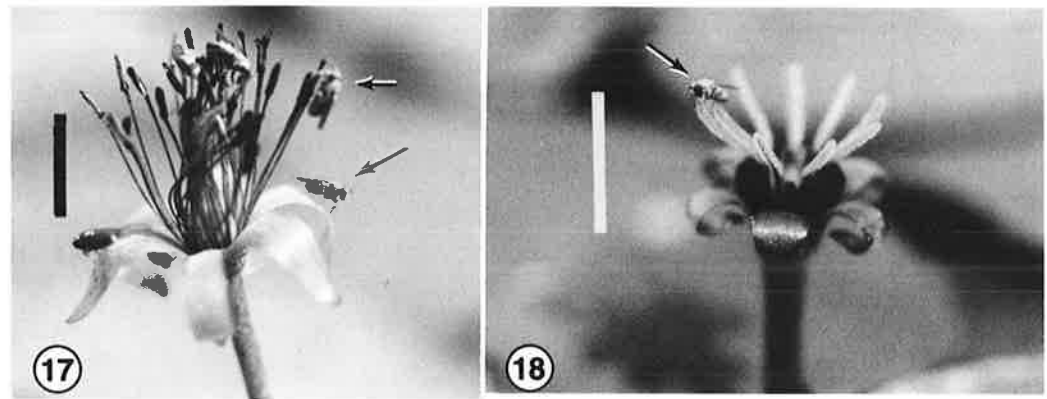
FIGURES 17, 18. *Insect visitors of* Brasenia.— *17. Post-dehiscent second-day flower with* Notiphila *cf.* cressoni *foraging for pollen.—18.* Notiphila *on stigma of first-day flower. Scale bars = 1 cm.*

the abundant airborne pollen that had become deposited on the adaxial surfaces of leaves. Microscopic examinations of dipteran bodies indicated that pollen loads were minimal except around the bases of the legs where body hairs are more dense. The stickiness of *Brasenia* pollen was not determined. Other insects were only occasional floral visitors and cannot be considered pollinators.

DISCUSSION

FLORAL CYCLE

Field observations of *Brasenia* confirm that anthesis is diurnal, with individual flowers opening and closing for two consecutive days. First-day flowers are morphologically pistillate; second-day flowers staminate. Caging experiments (Table 1) indicate that stigmas of first-day flowers are receptive to pollen and those of second-day flowers generally are not. Thus floral structure and function are correlated. Caging experiments further reveal that *Brasenia* flowers set seed only with pollen from an allogamous origin (sensu Faegri & van der Pijl, 1979). Although individual flowers are self-pollinating, seeds are not produced. It is unlikely that a genetic mechanism of self-incompatibility is responsible for the lack of seed set. Abundant seed production from geitonogamous pollinations is indicative of self-compatibility. In the populations studied, self-fertilization (autogamy) is prevented by dichogamy.

WIND POLLINATION

In the populations studied, *Brasenia* is predominantly anemophilous. This conclusion is reached by correlating data and observations of the following: pollinometer experiments, floral densities, number of viable pollen grains produced, flower position above water level, terminal settling velocity of pollen, and floral morphology. The pollinometer experiments reveal that *Brasenia* pollen is dispersed over a relatively short distance. Therefore, for successful pollination by wind, the flowers must be closely grouped and produce a significant amount of viable pollen. As has been shown, floral density can exceed 100 flowers/m² and viable pollen production > 200,000 grains/flower. The elevated position of second-day flowers above first-day flowers, together with a pollen terminal settling velocity of 7.7 cm/sec., further enhances the dispersal distance of *Brasenia* pollen. Direct observations of pollen on stigmas of first-day flowers downwind of pollen-releasing flowers at distances of < 1 m and > 2 m support the above conclusion. Large pollen–ovule ratios, as discovered in *Brasenia*, are also characteristic of wind-pollinated taxa (Faegri & van der Pijl, 1979).

Whitehead (1983) identified several "idealized conditions" that need to be met by a plant species if wind pollination is to be successful. The floral and vegetative morphologies of *Brasenia* are adapted for anemophily. Table 2 summarizes those adaptations of *Brasenia* in comparison with other anemophilous plants. Floral anomalies (e.g., short stigmas), however, are not as well adapted for reception of wind-borne pollen. This accounts for the low seed set in flowers with short stigmas (Table 1) compared with typical flowers.

Additionally, observations of infrequent insect visitations between first-day and second-day flowers, despite insect abundance, and minimal pollen loads negate entomophily as a primary mechanism of pollen transfer in this study site.

TABLE 2. *Comparison of* Brasenia *adaptations with Whitehead's "idealized conditions" for anemophily.*

Whitehead	*Brasenia*
There is production of large numbers of pollen grains.	Greater than 221,700 pollen grains are produced per average flower.
Pollen grains possess appropriate aerodynamic characteristics, are typically 20–40 μm in size, and have terminal settling velocities of 2-6 cm/sec.	Pollen grains are elliptic, smooth, within "ideal" size range ($36.3 \times 47.6 \times 36.9$ μm),[1] and have a terminal settling velocity of 7.7 cm/sec.
The probabilty of pollen's entrainment in moving air is maximized due to flower and inflorescence structure and their location on the plant.	Second-day (staminate) flowers possess elongated filaments that elevate dehiscent anthers; perianth parts are reflexed and stigmas are centrally aggregated out of the path of ambient air.
Stigmatic surfaces are structured and positioned to maximize collection efficiency.	First-day (pistillate) flowers possess elongated, papillate stigmas which radiate outward over the perianth providing an increased surface area for pollen adherence.
Pollen release is timed within both the season and the day to maximize the possibility of pollen capture by receptive conspecifics downwind.	Pistillate flowers are fully open and generally elevated lower than staminate flowers at the time of anther dehiscence.
There is a relatively close spacing of compatible plants.	Flowers exhibit relatively dense distribution, up to 110/m^2 during peak anthesis, and are both geitonogamous and xenogamous.
Plants possess a vegetational structure that is relatively open to minimize filtration of pollen by nonstigmatic surfaces.	Vegetative organs are either submerged or floating; the only emergent structures are floral.
The range of wind velocities ensures pollen transport and minimizes its downwind dispersion.	Pollen can be dispersed at least 70 cm at wind speeds as low as 1.6 kph.

[1] Ueno & Kitaguchi (1961).

OCCURRENCE OF INSECTS

The frequency, abundance, and behavior of *Notiphila* flies on second-day flowers and leaves are related to their life history and association with the other nymphaeaceous genera, *Cabomba* and *Nymphaea* (Van der Velde et al., 1978; Van der Velde & Brock, 1980; Willmer, 1982), in the study site. Members of *Notiphila,* while foraging on nectar and pollen, function as the primary pollinators of *Cabomba* (Schneider & Jeter, 1982). In *Nymphaea* the flies forage on pollen and occasionally sponge stigmatic fluid, but native halictid bees are pollinators of the diurnal Toledo Bend species of this genus.

We view the relationship of *Notiphila* with *Brasenia* as one of herbivory in which flies mainly forage for pollen and utilize the flower to groom and mate. The flies forage for pollen in anther sacs only after the majority of pollen grains have been wind dispersed and the inner UV reflective walls, which aid in the attraction of the flies, are exposed. As the flies move about the dehiscent androecium and pollen-covered perianth surface, a small quantity of pollen accumulates on their legs. This minute pollen load can be attributed at least in part to the smooth walls of the *Brasenia* pollen. Occasional visits to pistillate flowers may facilitate transfer of minimal pollen; therefore, *Notiphila* should only be considered an incidental pollinator. The occurrence of flies and their role as incidental pollinators, together with floral variations such as shortened stigmas, suggest a possible evolutionary shift from anemophily to partial myophily (ambophily; Stelleman, 1984).

POLLINATION BIOLOGY OF THE NYMPHAEACEOUS SENSU LATO FAMILIES

This investigation provides the first documentation of anemophily in the Nymphaeaceae s.l. Traditionally, wind pollination has been interpreted as derived in the angiosperms (Whitehead, 1969; Faegri & van der Pijl, 1979). Dilcher (1979), Dilcher & Kovach (1986), and Crane & Dilcher (1984), however, have shown that several extinct lower- to mid-Cretaceous flowering plants had the reproductive morphology to accommodate polli-

nation by both wind and insects. The occurrence of anemophily in the Nymphaeaceae s.l., a taxon traditionally viewed as primitive and entomophilous, dictates that the phylogeny of this group be re-examined.

Cabombaceae. Several studies (e.g., Kosakai, 1968; Goleniewska-Furmanowa, 1970; Bukowiecki et al., 1972; Okada & Tamura, 1981; Ito, 1986a, 1987) suggest *Cabomba* and *Brasenia* share sufficient characteristics to warrant maintenance of the Cabombaceae. From a classical viewpoint, it could be argued that the presence of perfect flowers, a perianth, the occurrence of foraging flies, and some UV reflectance in *Brasenia* are remnants of a former entomophilous condition which has more recently specialized for wind pollination. An alternative hypothesis, that anemophily is primitive, could also be argued. Support for this viewpoint comes from the fossil record in which Tertiary pollen of *Brasenia* exhibits the same morphological features as extant *Brasenia* pollen (Jessen et al., 1959). Although pollen alone is not a definitive indicator of pollination biology, modern *Brasenia* pollen is adapted for wind pollination (Table 2). Because fossil and extant pollen are morphologically similar, comparable functions can be postulated.

We do not view these two hypotheses as completely exclusive of each other. A third line of reasoning, that the cabombaceous ancestor had the reproductive morphology to facilitate both wind and insect pollination, could be advocated. An ancestor with dual pollination capabilities would explain the long anemophilous history as suggested by fossil pollen and the occurrence of the entomophilous features noted above. An extension of this third concept suggests that the pollination syndromes in extant *Brasenia* and *Cabomba* are specialized. Phylesis in *Cabomba*, therefore, would have involved a reduction and stabilization in the number of androecial and gynoecial members, as evidenced by the vasculature studies of Ito (1986a), and the appearance of a distinct, colorful perianth possessing nectaries to enhance a myophilous pollination syndrome (Schneider & Jeter, 1982). Here, pollination is achieved by the appropriate positions of the pollen-receptive stigmas in first-day flowers (Fig. 19L) and pollen-dehiscent anthers in second-day flowers (Fig. 19K) above the petaliferous nectaries where *Notiphila* flies sponge secretions. The elongated stigmatic surfaces which radiate above the recurved perianth in first-day flowers of *Brasenia* (Fig. 19N) enhance collection of airborne pollen. In second-day flowers (Fig. 19M), the el-

evation of anthers above the recurved perianth promotes pollen dispersal.

A recent cladistic analysis of the Nymphaeales by Ito (1987) indicates *Ceratophyllum* has association with *Brasenia* and *Cabomba*. Les (1986) has proposed that the hydrophilous pollination mechanism of *Ceratophyllum* (Jones, 1931) is derived from anemophily. The occurrence of wind pollination within the Cabombaceae warrants continued examination of the suggested relationships among *Ceratophyllum*, *Brasenia*, and *Cabomba*.

Nymphaeaceae s. str. Some investigations (Collinson, 1980; Moseley et al., 1984) indicate *Brasenia* shares more affinities with the Nymphaeaceae s. str. than with *Cabomba*. We do not support dismantling the Cabombaceae. It is our opinion that data from pollination biology, floral anatomy, anatomy of fossil and extant seeds, and embryology support a common ancestry hypothesis. The Nymphaeaceae s. str., unlike the Cabombaceae and Nelumbonaceae, possess completely syncarpous gynoecia. When correlated with an evolutionary shift from hypogyny in *Nuphar*, through perigyny in *Nymphaea*, to epigyny in *Barclaya*, *Euryale* (Kadono & Schneider, in press), and *Victoria*, flowers of this family exhibit an overall elaboration. This elaboration consists of an increase in size and number of floral parts and stigmatic surface area facilitated by radial extension of the carpels and by the appearance of specialized organs (e.g., staminodia and carpellary appendages). This family, like the Nelumbonaceae, is entomophilous except for the cleistogamous flowers of *Barclaya* (Fig. 19D), *Euryale* (Fig. 19C), and some species of *Nymphaea*.

The flowers of *Nuphar* (Fig. 19I, J) have a close relationship with beetles of the genus *Donacia*, which complete their life cycle in association with the plant, during which time they facilitate pollination (Schneider & Moore, 1977). Diurnal flowers of *Nymphaea* and *Ondinea* are specialized for washing pollen from the body of foraging native bees. This is accomplished by the production of stigmatic fluid on the first day of anthesis (Fig. 19F, H; Conard, 1905; Schmucker, 1933; Prance & Anderson, 1976; Schneider, 1979, 1982a, b, 1983; Meeuse & Schneider, 1980; Schneider & Chaney, 1981; Capperino & Schneider, 1985). The pollination biology of nocturnally flowering species of *Nymphaea* is largely unstudied, but cantharophily is known (Cramer et al., 1975; Wiersema, 1987).

Large, nocturnal flowers of *Victoria* are adapted for pollination by *Cyclocephala* beetles. Beetles
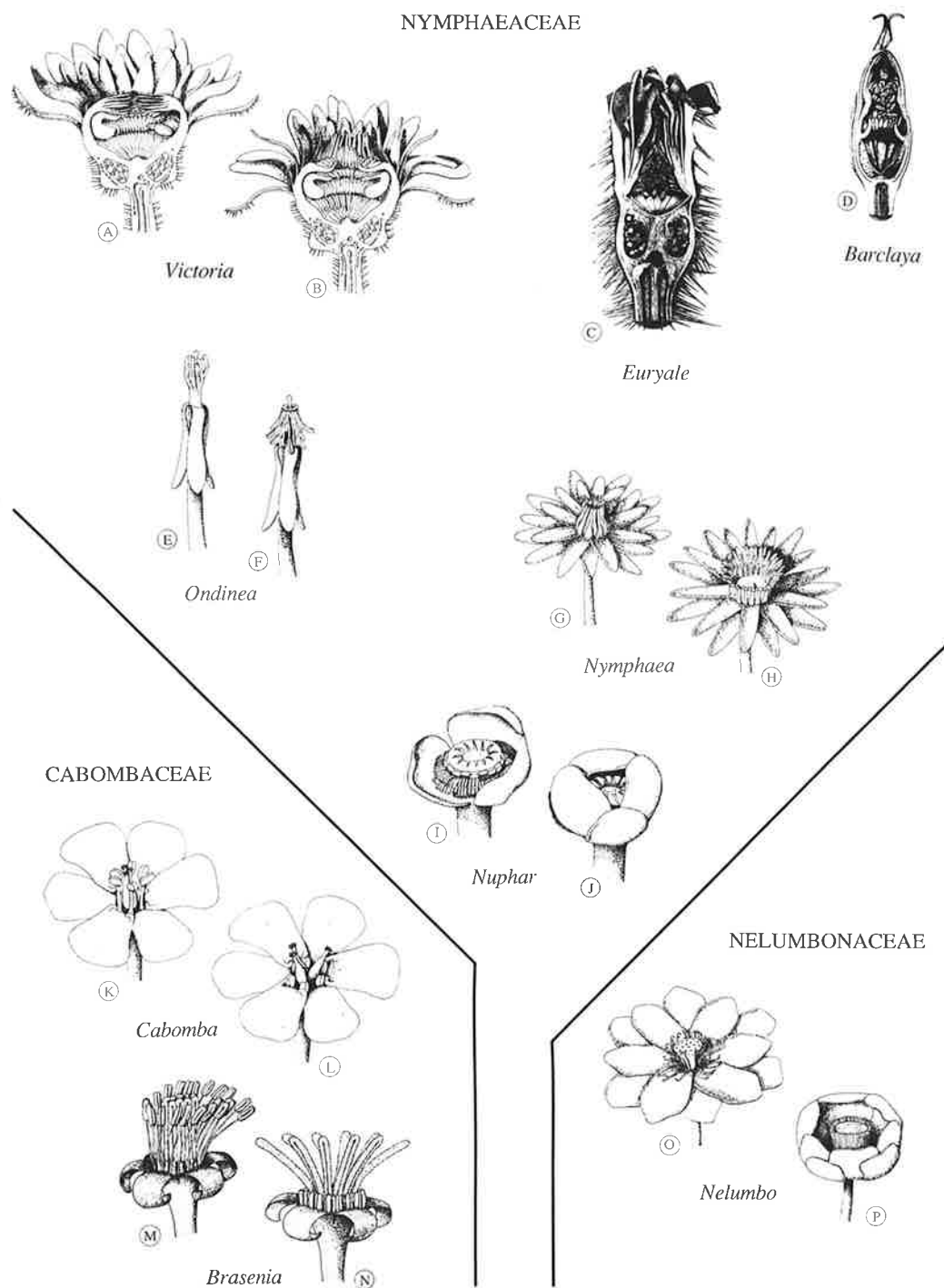
NYMPHAEACEAE

CABOMBACEAE

NELUMBONACEAE

FIGURE 19. *Comparative morphologies of first-day (pistillate) and second-day (staminate) flowers of the Cabombaceae (first-day, L, N; second-day, K, M), Nymphaeaceae s. str. (first-day, B, C, D, F, H, J; second-day, A, E, G, I), and Nelumbonaceae (first-day, P; second-day, O). Note: flowers not drawn to scale.*

are attracted by the white color and fragrance of first-night flowers. The diffusion of fragrance is accelerated by a rise in floral temperature due to the thermogenicity of carpellary appendages (Fig. 19B). Pollination is achieved when pollen-covered beetles crawl about the inner stigmatic cup. While "trapped" within the flower, beetles forage on starch-rich carpellary appendages (Fig. 19A). As beetles emerge from second-evening flowers they become dusted with pollen from the now-dehiscent anthers and, once again, are attracted to first-evening flowers (Knoch, 1899; Valla & Cirino, 1972; Prance & Arias, 1975; Schneider, 1976; Lovejoy, 1978).

Nelumbonaceae. Separation of *Nelumbo* from the Nymphaeaceae s.l. into the Nelumbonaceae has been justified on numerous grounds, including seed anatomy (Collinson, 1980) and floral anatomy/morphology (Moseley & Uhl, 1985; Ito, 1986b, 1987). Despite this separation, *Nelumbo* displays a floral behavior similar to that of chasmogamous flowers of the Cabombaceae and Nymphaeaceae s. str. in which first-day flowers are pistillate (Fig. 19P) and second-day flowers are staminate (Fig. 19O). Flowers of *Nelumbo* are principally adapted for cantharophily (Schneider & Buchanan, 1980), although bees and flies (Sohmer & Sefton, 1978) also transfer pollen. Moseley & Uhl (1985) suggested that the flower of *Nelumbo* represents an evolutionary elaboration in response to cantharophily.

Comparative studies of the reproductive biology of Cabombaceae, Nymphaeaceae s. str., and Nelumbonaceae, when viewed in conjunction with structural aspects of the flower, suggest that evolution within these families has involved both floral reduction and elaboration as a result of adaptive evolution in response to abiotic and biotic vectors. This concept is consistent with other angiosperm taxa as indicated by the fossil record (Basinger & Dilcher, 1984).

LITERATURE CITED

ADAMS, F. S. 1969. Winterbud production and function in *Brasenia schreberi*. Rhodora 71: 417–433.

ANDERSEN, A. A. 1958. A new sampler for the collection, sizing and enumeration of viable airborne bacteria. J. Bacteriol. 76: 471–484.

BASINGER, J. F. & D. L. DILCHER. 1984. Ancient bisexual flowers. Science 224: 511–513.

BATYGINA, T. B. & I. I. SHAMROV. 1983. Embryology of the Nelumbonaceae and Nymphaeaceae: pollen grain structure. Bot. Žurn. (Moscow & Leningrad) 68: 1177–1183. [In Russian.]

BENTHAM, G. & J. D. HOOKER. 1862. Genera Plantarum, Volume I. Reeve & Co., London.

BUKOWIECKI, H., M. FURMANOWA & H. OLEDZKA. 1972.

The numerical taxonomy of Nymphaeaceae Bentham et Hooker. I. Estimation of taxonomic distance. Acta Polon. Pharm. 29: 319–327.

——, & ——. 1974. The numerical taxonomy of Nymphaeaceae Bentham et Hooker. II. Estimation of similarity coefficients. Acta Polon. Pharm. 31: 385–391.

CAPPERINO, M. E. & E. L. SCHNEIDER. 1985. Floral biology of *Nymphaea mexicana* Zucc. (Nymphaeaceae). Aquatic Bot. 23: 83–93.

CASPARY, R. 1891. Nymphaeaceae. *In:* A. Engler & K. Prantl, Die Natürlichen Pflanzenfamilien 3: 1–10.

CHEN, W. & S. ZHANG. 1986. Peltate leaf development in *Brasenia schreberi*. Acta Bot. Sin. 28: 341–348. [In Chinese with English summary.]

CHIFFLOT, J. B. J. 1902. Contributions à l'étude de la classe des Nymphéinées. Ann. Univ. Lyon, N.S. 1 Sci. Med. 10: 19–38.

CHRYSLER, M. A. 1938. The winter buds of *Brasenia*. Bull. Torrey Bot. Club 65: 277–283.

CLARK, G. C. & M. R. JONES. 1981. The northwest European pollen flora 24. Cabombaceae. Rev. Palaeobot. Palynol. 33: 51–55.

COLLINSON, M. E. 1980. Recent and Tertiary seeds of the Nymphaeaceae sensu lato with a revision of *Brasenia ovula* (Brong.) Reid and Chandler. Ann. Bot. 46: 603–632.

CONARD, H. S. 1905. The water lilies. A monograph of the genus *Nymphaea*. Publ. Carnegie Inst. Wash. 4: 119–123.

COOK, M. T. 1906. The embryogeny of some Cuban Nymphaeaceae. Bot. Gaz. (Crawfordsville) 42: 376–392.

———. 1909. Notes on the embryology of the Nymphaeaceae. Bot. Gaz. (Crawfordsville) 48: 56–61.

CORNER, E. J. H. 1976. The Seeds of Dicotyledons, Volumes 1, 2. Cambridge Univ. Press, New York.

CRAMER, J. M., A. D. J. MEEUSE & P. TEUNISSEN. 1975. A note on the pollination of nocturnally flowering species of *Nymphaea*. Acta Bot. Neerl. 24: 489–490.

CRANE, P. R. & D. L. DILCHER. 1984. *Lesqueria*: an early angiosperm fruiting axis from the mid-Cretaceous. Ann. Missouri Bot. Gard. 71: 384–402.

CRONQUIST, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia Univ. Press, New York.

CRUDEN, R. W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

DEN HARTOG, A. 1970. *Ondinea purpurea*, a new genus for the Nymphaeaceae. Blumea 18: 413–417.

DILCHER, D. L. 1979. Early angiosperm reproduction: an introductory report. Rev. Paleobot. Palynol. 27: 291–328.

——— & W. L. KOVACH. 1986. Early angiosperm reproduction: *Caloda delevoryana* gen. et sp. nov., a new fructification from the Dakota formation (Cenomanian) of Kansas. Amer. J. Bot. 73: 1230–1237.

DOROFEEV, P. I. 1984. The taxonomy and history of the genus *Brasenia* (Cabombaceae). Bot. Žurn. (Moscow & Leningrad) 69: 137–148. [In Russian.]

ESAU, K. 1965. Plant Anatomy, 2nd edition. John Wiley and Sons, New York.

FAEGRI, K. & L. VAN DER PIJL. 1979. The Principles of Pollination Ecology, 3rd edition. Pergamon Press, New York.

GOLENIEWSKA-FURMANOWA, M. 1970. Comparative leaf anatomy and alkaloid content in the Nymphaeaceae Bentham and Hooker. Monogr. Bot. 31: 5–54.

GWYNNE-VAUGHN, D. T. 1897. On some points in the morphology and anatomy of the Nymphaeaceae. Trans. Linn. Soc. London, Bot. 5: 287–299.

HALL, A. 1978. Some new paleobotanical records for the British Ipswichian interglacial. New Phytol. 81: 805–812.

HILL, E. J. 1900. An observation on the water-shield (*Brasenia peltata*). Pl. World 3: 153.

IKUSE, M. 1955. General survey list of pollen grains in Japan (3). J. Jap. Bot. 30: 102–113. [In Japanese.]

ITO, M. 1986a. Studies in the floral morphology and anatomy of Nymphaeales III. Floral anatomy of *Brasenia schreberi* Gmel. and *Cabomba caroliniana* A. Gray. Bot. Mag. (Tokyo) 99: 169–184.

———. 1986b. Studies in the floral morphology and anatomy of Nymphaeales IV. Floral anatomy of *Nelumbo nucifera*. Acta Phytotax. Geobot. 37: 82–96.

———. 1987. Phylogenetic systematics of the Nymphaeales. Bot. Mag. (Tokyo) 100: 17–35.

JESSEN, K., S. TH. ANDERSEN & A. BARRINGTON. 1959. The interglacial deposit near Gort, Co., Galway, Ireland. Proc. Roy. Irish Acad. 60: 1–78.

JONES, E. N. 1931. The morphology and biology of *Ceratophyllum demersum*. Stud. Nat. Hist. Iowa Univ. 13: 11–55.

KADONO, Y. & E. L. SCHNEIDER. The life history of *Euryale ferox* Salisb. in southwestern Japan with special reference to reproductive ecology. Pl. Sp. Biol. 2 (in press).

KAKUTA, M. & A. MISAKI. 1979. The polysaccharide of "junsai" (*Brasenia schreberi* J. F. Gmel.) mucilage; fragmentation analysis by successive Smith degradations and partial acid hydrolysis. Agric. Biol. Chem. 43: 1269–1276.

KAUL, R. B. 1976. Anatomical observations on floating leaves. Aquatic Bot. 2: 215–234.

KELLER, I. A. 1893. The glandular hairs of *Brasenia peltata* Pursh. Proc. Acad. Nat. Sci. Philadelphia: 188–193.

KHANNA, P. 1965. Morphological and embryological studies in the Nymphaeaceae. II. *Brasenia schreberi* Gmel. and *Nelumbo nucifera* Gaertn. Austral. J. Bot. 13: 379–387.

KNOCH, E. 1899. Untersuchungen über die Morphologie, Biologie und Physiologie der Blüte von *Victoria regia*. Biblioth. Bot. 9: 1–67.

KOSAKAI, H. 1968. The Comparative Xylary Anatomy of Various Nymphaeaceae. M.A. Thesis. University of California, Santa Barbara, California.

KRISTEN, U. 1974. Zur Feinstruktur der submersen Drüsenpapillen von *Brasenia schreberi* und *Cabomba caroliniana*. Cytobiologie 9: 36–44.

LANGLET, O. & E. SÖDERBERG. 1927. Über die Chromosomenzahlen einiger Nymphaeaceen. Acta Horti Berg. 9: 85–104.

LAWSON, G. 1888. On the Nymphaeaceae. Proc. & Trans. Roy. Soc. Canad. 6: 97–123.

LES, D. H. 1986. The origin and affinities of the Ceratophyllaceae. Amer. J. Bot. 73: 773–774. [Abstract.]

LINN, C. H., R. H. FALK & C. R. STOCKING. 1977. Rapid chemical dehydration of plant material for light and electron microscopy with 2,2-dimethoxypropane and 2,2-diethoxypropane. Amer. J. Bot. 64: 601–605.

LOVEJOY, T. E. 1978. Royal water lilies, truly amazonian. Smithsonian 9: 78–84.

MATSUDA, T. & H. HARA. 1985. On the problems related to the cultivation of water-shield (*Brasenia schreberi*) at the reclaimed land of Haga Swamp (Japan). Sci. Rep. Fac. Agric. Ibaraki Univ. 33: 1–13. [In Japanese.]

MEEUSE, B. J. D. & E. L. SCHNEIDER. 1980. *Nymphaea* revisited: a preliminary communication. Israel J. Bot. 28: 65–79.

MEIER, N. R. 1964. Palynological studies of Nymphaeaceae. Bot. Žurn. (Moscow & Leningrad) 49: 1421–1429. [In Russian.]

MELIKYAN, A. P. 1964. Histogenesis of the seed coat in *Brasenia schreberi* and *Nymphaea capensis*. Vestn. Leningradsk. Univ., Ser. Biol. 2: 121–125. [In Russian.]

MOSELEY, M. F. 1958. Morphological studies of the Nymphaeaceae. I. The nature of the stamens. Phytomorphology 8: 1–29.

——— & N. W. UHL. 1985. Morphological studies of the Nymphaeaceae sensu lato. XV. The anatomy of the flower of *Nelumbo*. Bot. Jahrb. Syst. 106: 61–98.

———, I. J. MEHTA, P. S. WILLIAMSON & H. KOSAKAI. 1984. Morphological studies of the Nymphaeaceae sensu lato. XIII. Contributions to the vegetative and floral structure of *Cabomba*. Amer. J. Bot. 71: 902–924.

NAKAHARA, H. 1940. Über den Schleim von *Brasenia schreberi* Gmel. Agric. Chem. Soc. Japan J. 16: 140–141.

NIKLAS, K. J. 1984. The motion of windborne pollen grains around conifer ovulate cones: implications on wind pollination. Amer. J. Bot. 71: 356–374.

OGDEN, E. C. 1974. Anatomical patterns of some aquatic vascular plants of New York. New York State Mus. Sci. Serv. Bull. 424: 1–133.

OKADA, H. & M. TAMURA. 1981. Karyomorphological study of the Nymphaeales. J. Jap. Bot. 56: 367–375.

POSTEK, M. T. & S. C. TUCKER. 1976. A new short chemical dehydration method for light microscopy preparations of plant material. Canad. J. Bot. 54: 872–875.

PRANCE, G. T. & A. B. ANDERSON. 1976. Studies on the floral biology of neotropical Nymphaeaceae. 3. Acta Amazonica 6: 163–170.

——— & J. R. ARIAS. 1975. A study of the floral biology of *Victoria amazonica* (Poepp.) Sowerby (Nymphaeaceae). Acta Amazonica 5: 109–139.

RACIBORSKI, M. 1894. Die Morphologie der Cabombeen und Nymphaeaceen. Flora 78: 244–279.

RAO, T. A. & B. C. BANERJEE. 1979. On foliar sclereids in the Nymphaeaceae sensu lato and their use in familial classification. Proc. Indian Acad. Sci. 88B: 413–422.

RICHARDSON, F. C. 1969. Morphological studies of the Nymphaeaceae. IV. Structure and development of the flower of *Brasenia schreberi* Gmel. Univ. Calif. Publ. Bot. 47: 1–101.

RIEMER, D. N. & S. J. TOTH. 1970. Chemical composition of five species of Nymphaeaceae. Weed Sci. 18: 4–6.

SCHILLING, A. J. 1894. Anatomisch-biologische Untersuchungen über die Schleimbildung der Wasserpflanzen. Flora 78: 280–360.

SCHMUCKER, T. 1933. Zur Blütenbiologie tropischer

Nymphaea-Arten. II. Bor als entscheidender Faktor. Planta 18: 641–650.

SCHNEIDER, E. L. 1976. Morphological studies of the Nymphaeaceae. VIII. The flower of *Victoria* Schomb. J. Linn. Soc., Bot. 72: 115–148.

———. 1978. Morphological studies of the Nymphaeaceae. X. The seed of *Ondinea purpurea* den Hartog. Bull. Torrey Bot. Club 105: 192–200.

———. 1979. Pollination biology of the Nymphaeaceae. Proc. IVth Int. Symp. on Pollination, Md. Agric. Exp. Sta. Special Misc. Publ. 1: 419–429.

———. 1982a. The floral biology of *Nymphaea elegans* (Nymphaeaceae) in Texas. Aquatic Bot. 12: 197–200.

———. 1982b. Observations on the pollination biology of *Nymphaea gigantea* W. J. Hooker (Nymphaeaceae). Western Austral. Naturalist 15: 71–72.

———. 1983. Morphological studies of the Nymphaeaceae. XIV. Gross morphology and floral biology of *Ondinea purpurea* den Hartog. Austral. J. Bot. 31: 371–382.

——— & J. D. BUCHANAN. 1980. Morphological studies of the Nymphaeaceae. XI. The floral biology of *Nelumbo pentapetala*. Amer. J. Bot. 67: 182–193.

——— & T. CHANEY. 1981. The floral biology of *Nymphaea odorata* (Nymphaeaceae). Southw. Naturalist 26: 159–165.

——— & J. M. JETER. 1982. Morphological studies of the Nymphaeaceae. XII. The floral biology of *Cabomba caroliniana*. Amer. J. Bot. 69: 1410–1419.

——— & L. A. MOORE. 1977. Morphological studies of the Nymphaeaceae. VII. The floral biology of *Nuphar lutea* subsp. *macrophylla*. Brittonia 29: 88–99.

SCHRENK, J. 1888. On the histology of the vegetative organs of *Brasenia peltata* Pursh. Bull. Torrey Bot. Club 15: 28–47.

SEVILLA, F., L. A. DEL RIO & E. HELLIN. 1984. Superoxide dismutases from a *Citrus* plant. J. Pl. Physiol. 116: 381–388.

SOHMER, S. H. & D. F. SEFTON. 1978. The reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) on the upper Mississippi River. II. The insects associated with the transfer of pollen. Brittonia 30: 355–364.

SRODON, A. 1935. Studies of diluvial plant remains of the family Nymphaeaceae. Starunia 7: 1–10. [In Polish.]

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

TAKHTAJAN, A. 1980. Outline for the classification of flowering plants (Magnoliophyta). Bot. Rev. 46: 225–359.

TOKURA, A. 1937. On the blooming of *Brasenia*. Jap. J. Bot. 13: 51–61. [In Japanese.]

TRALAU, H. 1959. Extinct aquatic plants of Europe. Bot. Not. 112: 385.

TROLL, W. 1933. Beiträge zur Morphologie des Gynaeceums. IV. Über das Gynaeceum der *Nymphaeaceen*. Planta 21: 447–485.

UENO, J. 1962. On the fine structure of the pollen walls of Angiospermae. II. *Victoria*. J. Biol. Osaka City Univ. 13: 99–104.

——— & S. KITAGUCHI. 1961. On the fine structure of the pollen walls of Angiospermae. I. Nymphaeaceae. J. Biol. Osaka City Univ. 12: 83–90.

VALLA, J. J. & D. R. CIRINO. 1972. Biologia floral del irupé, *Victoria cruziana* D'Orb. (Nymphaeaceae). Darwiniana 17: 447–500.

VAN DER VELDE, G. & TH. C. M. BROCK. 1980. The life history and habits of *Notiphila brunnipes* Robineau-Desvoidy (Diptera, Ephydridae), an autecological study on a fly associated with nymphaeid vegetations. Tijdschr. Entomol. Deel 123: 105–127.

———, ———, M. HEINE & P. M. PEETERS. 1978. Flowers of Dutch Nymphaeaceae as a habitat for insects. Acta Bot. Neerl. 27: 429–430.

VINOGRADOV, I. S. 1967. Sistema semeistva *Nymphaeaceae* na osnove analiza morfologicheskogo stroya. Zap. Centr.-Kavkazsk. Otd. Vsesojuzn. Bot. Obšč. 2: 5–11.

VOGEL, S. 1966. Scent organs of orchid flowers and their relation to insect pollination. Proc. 5th World Orchid Conf., Long Beach, California: 253–259.

WALKER, J. W. 1976a. Comparative pollen morphology and phylogeny of the ranalean complex. *In:* C. B. Beck (editor), Origin and Early Evolution of Angiosperms. Columbia Univ. Press, New York.

———. 1976b. Evolutionary significance of the exine in the pollen of primitive angiosperms. *In:* I. K. Ferguson & J. Muller (editors), The Evolutionary Significance of the Exine. Linn. Soc. Symp. Ser. 1: 251–308.

——— & J. A. DOYLE. 1975. The bases of angiosperm phylogeny: palynology. Ann. Missouri Bot. Gard. 62: 664–723.

WEBERBAUER, A. 1893. Über die fossilien Nymphaeaceen Gattungen *Holopleura* und *Cratopleura* Web. und ihre Beziehungen zu der rezenten Gattung *Brasenia*. Ber. Deutsch. Bot. Ges. 11: 336–374.

———. 1894. Beiträge zur Samenanatomie der Nymphaeaceen. Bot. Jahrb. Syst. 18: 213–258.

WHITEHEAD, D. R. 1969. Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution 23: 28–35.

———. 1983. Wind pollination: some ecological and evolutionary perspectives. *In:* L. Real (editor), Pollination Biology. Academic Press, Orlando, Florida.

WIERSEMA, J. H. 1987. A monograph of *Nymphaea* subgenus *Hydrocallis* (Nymphaeaceae). Syst. Bot. Monogr. 16: 1–112.

WILLMER, P. G. 1982. Hygrothermal determinants of insect activity patterns: the Diptera of water-lily leaves. Ecol. Entomol. 7: 221–231.

WODEHOUSE, R. P. 1932. Tertiary pollen. I. Pollen of the living representatives of the Green River flora. Bull. Torrey Bot. Club 59: 313–340.

WOOD, C. E. 1959. The genera of the Nymphaeaceae and Ceratophyllaceae in the southeastern United States. J. Arnold Arbor. 40: 94–112.