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Pollination of *Nuphar* (*Nymphaeaceae*) in Europe: flies and bees rather than *Donacia* beetles

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Abstract: *Nuphar* (*Nymphaeaceae*) comprises a small holarctic group of aquatic perennials whose flowers are pollinated by flies, bees, and beetles. We studied pollination in different populations of the two European species *N. lutea* and *N. pumila* in Norway and in Germany. Flowers are self-compatible and protogynous, preventing automatic selfing, and insect pollination is required for seed production. Sympatric populations were studied in Vest-Agder county in Norway to determine whether *N. lutea* and *N. pumila* have the same or different pollinators. Allopatric populations of *N. lutea* in Germany and Norway were then compared to determine whether their pollinator spectra differ as would be expected in an open flower with seemingly easily accessible pollen and nectar. Results of the present as well as previous studies of the pollination of *N. lutea* and *N. pumila* show that both species are mainly pollinated by flies, including apparent *Nuphar* specialists, such as the scatophagid *Hydromyza livens* and the ephydrids *Hydrellia* and *Notiphila*, the last also a long-known pollinator of *N. advena* in Florida. Pollinator overlap between sympatric heterospecific populations was small, while allopatric conspecific populations had similar visitor and pollinator spectra. We found no evidence of pollination by *Donacia* beetles as reported from some North American populations of *Nuphar*.

Yellow water lilies, *Nuphar lutea* (L.) SM. and congeners, are aquatic perennials distributed throughout the temperate Northern Hemisphere that have long attracted the interest of systematists and ecologists. Systematists have found the extreme morphological variability of yellow water lilies difficult to deal with, which has resulted in continuing disagreement about the number of species in the genus, with between two and 20 species being recognized worldwide (HESLOP-HARRISON 1955; BEAL 1956; MEUSEL & MÜHLBERG 1965; COOK 1990; STACE 1991; WIERSEMA & HELLQUIST 1997; D. J. PADGETT, pers. comm.). Ecologists have been intrigued by the association of certain chrysomelid beetles (*Donacia* s. str.: *Donaciinae*) with their nymphaeaceous host plants, which appears to go back at least 60 million years (CROWSON 1981; ASKEVOLD 1990, 1991), as well as by the pollination syndrome of

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* Dedicated to Univ.-Prof. Dr F. EHRENDORFER on the occasion of his 70th birthday

Nuphar, where discussion centers on whether or not all or only some species of *Nuphar* are also pollinated by these beetles (ROBERTSON 1889, SCHNEIDER & MOORE 1977, SCHNEIDER 1979, VAN DER VELDE 1986, SCHNEIDER & WILLIAMSON 1993, ERVIK & al. 1995).The *Donaciinae* are of pre-Tertiary origin and are a diverse taxon of which the most derived group, *Donacia* s. str., feeds and oviposits exclusively on *Nymphaea* and *Nuphar* leaves.

Recent discussions of *Nuphar* floral biology state that "the flowers have a close relationship with beetles of the genus *Donacia*, which complete their life cycle in association with the plant, during which time they facillitate pollination" (SCHNEIDER & WILLIAMSON 1993) and "[···] the unique aquatic adaptations of *Donacia* (e.g., caudal spine) and the specificity which the beetle shows to *Nuphar* are suggestive of a long coevolutionary pollination process" (SCHNEIDER 1979). These statements are based on a study conducted by SCHNEIDER & MOORE (1977; see also SCHNEIDER 1979: fig. 2) on *N. advena* (AIT.) AIT. f. [= *N. lutea* subsp. *macrophylla* (SMALL) BEAL] in central Texas where *D. piscatrix* LAC. visits and pollinates the flowers. Additional effective pollinators were *Apis mellifera* and halictid bees, but SCHNEIDER & MOORE (1977) felt that *Donacia* were more effective "because of their abundance and the length of time they remain in the flowers, whereas the bees soon departed." An average of three beetles, carrying copious amounts of pollen, was found in 32 first-night (female stage) flowers. Beetles were also found in older, male stage flowers where they must have picked up their pollen loads because the flowers are strongly protogynous. VERNE GRANT in California and B. J. D. MEEUSE in-Washington (pers. comms. in SCHNEIDER & MOORE 1977) also found *Donacia*, namely *D. pusilla* SAY [*Plateumaris pusilla* (SAY)] and *D. proxima* (KIRBY), visiting flowers of *N. polysepala* ENGELM. in addition to syrphid flies and bees. While the usual hosts of *D. pusilla* are *Cyperaceae* (ASKEVOLD 1988, 1990), *D. proxima* and *D. piscatrix*, the species observed by SCHNEIDER & MOORE, both specialize on *Nymphaeaceae*. Based on these and their own observations, SCHNEIDER & MOORE (1977) interpreted floral structure in *Nuphar*, viz. the numerous stamens with their abundant pollen and the flat to somewhat concave stigmatic disk as well as the nocturnal closing of the flowers and the intense sweet scent, as "primary adaptions to assure polination by beetles" and they went on to suggest that "the evolution of other insects (e.g., bees) and other aquatic plants [···] brought about the appearance of new pollinators of *Nuphar* and the adaptive radiation of *Donacia*."

This interpretation of the floral syndrome of *Nuphar*, however, is in contrast to other observations. SPRENGEL (1793: 274 and plate 23, fig. 5) reported that *Meligethes* beetles were visiting flowers of *N. lutea* near Berlin, but he felt that "ein anderes mir noch unbekanntes Insekt" might (also?) pollinate the flowers. And ROBERTSON (1889), who observed sweat bees and syrphid flies pollinating *N. advena* in Illinois and ephydrid flies (*Notiphila* sp.) and *D. piscatrix* pollinating *N. advena* in Florida, regarded the beetles as "worse than useless when it comes to pollination." KNUTH (1898: 62–63) observed *Donacia* and *Meligethes* as well as flies of the genera *Scatophaga* (*Hydromyza*), *Notiphila*, *Calliphora*, and *Onesia* on *Nuphar* in northern Germany, but does not discuss their individuals roles. Modern studies of European *N. lutea* populations by VAN DER VELDE (1986) and ERVIK & al. (1995) in the Netherlands and in Norway, respectively, did not find the flowers to be pollinated by *Donacia*.

To address the questions of (1) pollination specialization and (2) the significance of *Donacia* beetles as pollinators of *Nuphar*, we studied the pollination of the two European species, *N. lutea* and *N. pumila* (TIMM) DC., in Germany and Norway. Specifically, we focussed on the roles of different insects and on the breeding system in order to assess to what extent these species depend on insect-mediated outcrossing. Our goal was (1) to compare allopatric populations of the same species to see whether they would be pollinated by different pollinators, and (2) to compare sympatric populations of the two species to determine whether floral morphological differences between them are associated with different pollinators, for example, by excluding or attracting particular insects. *Nuphar* has open, bowl-shaped flowers with seemingly easily accessible pollen and nectar rewards; such flowers might simply sample locally available insects. Both goals could be achieved by studying geographically close populations of *N. pumila* and *N. lutea* in Norway and by then comparing the Norwegian populations of *N. lutea* with southern German populations of *N. lutea*. *Nuphar pumila* flowers differ from those of *N. lutea* mainly in being smaller, having fewer petals and stamens, lacking staminodes, and having fewer stigmatic rays. They also differ in a number of physiological characters, such as nocturnal flower closure and duration of nectar presence. The German populations of *N. lutea* were located some 1000 km south of the Norwegian study populations of this species, while the Norwegian populations of *N. lutea* and *N. pumila* were located within 100 km of each other.

Materials and methods

Study sites. Observations on *N. lutea* in southwest Germany were made by BL on plants growing in two oxbow lakes parallel to the Rhine near Bingen (elevation 80 m a.s.l.; lat. 49°59′ N, long. 7°52′ E) between July 21 and August 10, 1994. Each lake covered approximately 10000 m^2, with a maximum depth of 3 m. The lakes are regularly flooded when the water table is high, and their margins are covered with soft mud. Associated with the yellow water lilies were *Myriophyllum* and *Potamogeton* spp. Observations on Norwegian populations of *N. lutea* were made in three fresh-water lakes in Vest-Agder county (elevation 111 m; lat. c. 58° N, long. 7°30′ E) in the southernmost part of Norway by F. ERVIK between June 25 and August 7, 1992.

Observations on *N. pumila* were made by BL in the mountain lake Svartevatn and small ponds nearby (elevation 554 m; lat. 58°55′ N, long. 6°52′ E) in the valley of Sirdal also in Vest-Agder county between July 16 and August 9, 1995. Svartevatn covers approximately 270000 m^2 with a maximum depth of 20 m. It has no major inlets or outlets; the water is acidic and clear, and the ground around it is covered with peat. Associated species were *Sparganium* sp. and *Lobelia dortmanna* L.

The plants. The only European species of *Nuphar* are *N. lutea* and *N. pumila*, the recognition of the apparent hybrid *N. spenneriana* GAUDIN being controversial. Several species occur in North America. *Nuphar lutea* produces a creeping rhizome with two types of leaves, coriaceous ones that float on the surface and translucent and crumpled ones that stay submerged. The floating leaves may reach 30 cm in length and 26 cm in width, with more or less rounded petioles and overlapping or closely adjacent lobes. The axillary, bright yellow flowers measure about 4 cm in diam. and are borne on rigid peduncles about 3–5 cm above the water surface. They have five broadly spatulate sepals, which may be tinged green on the outside, and, on average, 15 petals, each with a dorsobasal (i.e. abaxial) nectary. The c. 140 (N = 5 flowers) 10 mm long, oblong-linear stamens are packed below the stigmatic disk before anthesis and open centripetally. There is a gradation in outward direction from conventional stamens to petaloid stamens, staminoid petals, and petals. Each anther contains about 1500 pollen grains (N = 1 anther), each c. 70 µm in length. The ovary is bottle-shaped and is crowned by a circular flattened disk with typically 17 rays of stigmatic tissue that do not reach the margin of the disk. The fruits contain numerous c. 5 mm long, ovoid, dark olive-green seeds. On average there were 300 ovules per ovary (N = 3 ovaries).

Nuphar pumila consists of smaller plants than *N. lutea.* The floating leaves are at most 17 cm long and 12 cm wide, with compressed, usually reddish-brown petioles, and divergent lobes. The flowers are about 2.5 cm in diam., have five slightly obovate sepals, and on average 11 petals rather than 15 as in *N. lutea.* The yellow of the floral parts is less intense than in *N. lutea* and a greater part of the outside of the sepals is tinged green. No petaloid stamens are found between the petals and the c. 48 stamina (N = 5 flowers). Each anther contains about 1460 pollen grains (N = 1 anther), each c. 55 µm in length and thus smaller than the grains of *N. lutea.* The stigma has eight, rather than 17, rays of stigmatic tissue and these reach the margin of the deeply dentate (rather than circular) stigmatic disk. The neck of the bottle-shaped fruit is more ridged than it is in *N. lutea.* On average there were 110 ovules per ovary (N = 3 ovaries).

Stigmatic secretion and nectar were tested qualitatively for glucose with diabetes test paper, and intact flowers of different ages were tested for terpenoid content indicative of floral scent production by immersion in a watery solution of neutral red (VOGEL 1990).

Insect visitation. In 1994, one of us (BL) monitored the German study population of *N. lutea* for a total of 37 h during 20 days and for 6 h during one night. Insect visitation rates were quantified during 1 h periods between 9 a.m.–1 p.m. on 12 of the 20 days and between 1–6 p.m. on the remaining 8 days. Flowers of the Norwegian population of *N. pumila* were monitored between 8 a.m.–12 p.m. (with interruptions) for a total of 20 h during 10 days and for 4 h during one night in 1995 (BL). Norwegian *N. lutea* was monitored between 8 a.m.–6 p.m. (with interruptions) for a total of 50 h during 20 days and for 5 h during one night. Representative specimens of all insect morphospecies were collected for identification; for this, bees and large flies were mounted and small flies and beetles were kept in 70% alcohol. Dried insects were latter examined under a scanning electron microscope to check for adherent pollen.

Evaluation of the breeding system. We examined the effects of six pollination treatments (Table 1) on both species in Germany and in Norway. For five of these treatments, healthy pre-anthetic flowers were covered with cloth mesh bags large enough to allow regular unfolding of the flowers, but excluding insects. In the sequence of Table 1, the treatments were (1) emasculation as a test for agamospermy; (2) bagging without further manipulation as a test for automatic selfing; (3) emasculation and hand selfing of 2nd-day flowers (1st day flowers can not be selfed because the flowers are protogynous; see Results); (4) emasculation and hand outcrossing of 1st-day flowers with pollen from plants from ponds 50 m to 1.5 km away; and (5) emasculation and hand outcrossing of 2nd-day flowers. The sixth treatment consisted of the emasculation of freshly opended flowers to assess natural levels of cross pollination or insect-mediated geitonogamy (if ramets had several open flowers). Several randomly chosen stalks were left untouched to serve as naturally pollinated controls. For the cross pollinations, anthers were rubbed onto the stigmatic disks until pollen grains could be seen to adhere to the stigmatic rays. Fruits were collected and dissected to determine seed set.

Results

Breeding system. Mean numbers of seed produced in the two species from the six pollination treatments are presented in Table 1. The table also shows the respective

Table 1. Numbers of seeds per capsule produced after experimental treatments of flowers of *Nuphar lutea* in Germany and Norway, and of *N. pumila* in Norway

	N. lutea in Germany		*N. lutea* in Norway[a]		*N. pumila* in Norway	
Treatment	Mean ± SE	(n)	Mean ± SE	(n)	Mean ± SE	(n)
Emasculation	0	(15)	0	(3)	0	(12)
Automatic selfing	0	(15)	0	(20)	0	(12)
Hand selfing of 2nd-day flowers	151 ± 78	(20)	204 ± 120	(21)	11 ± 3	(10)[b]
Hand outcrossing of 1st-day flowers	182 ± 70	(18)	161 ± 93	(12)	n.a.	
Hand outcrossing of 2nd-day flowers	127 ± 102	(18)	183 ± 143	(7)	20 ± 17	(5)
Natural pollination	194 ± 67	(17)	161 ± 93	(12)	15 ± 3	(5)
Control	265 ± 88	(20)	244 ± 89	(30)	46 ± 39	(22)

[a] From Ervik & al. (1995)

[b] Most of the bagged flowers of *N. pumila* drowned for two days just after the experimental treatments due to exceptionally heavy rains, which probably caused some of the pollen applied to the stigmas to be washed away.

results for *N. lutea* in Norway for comparision with seed set in this species in Germany. Due to exceptionally heavy rains most of the bagged flowers of *N. pumila* drowned for two days just after the experimental treatments. This may have washed away some of the pollen applied to the stigmas, which would explain the low seed set.

Neither species is agamospermous or selfs automatically. Experimental selfing of both species resulted in an only slightly lower seed-set than did experimental outcrossing, indicating that *N. lutea* is facultatively autogamous when self pollen is deposited on the stigmas by insects (Table 1). Receptivity of first-day flowers of *N. lutea* was significantly higher than that of second-day flowers (Table 1). Naturally pollinated flowers had a seed set similar to that of experimentally cross-pollinated first-day flowers.

The mean pollen-ovule ratio of three *N. lutea* flowers was 707 and that of three *N. pumila* flowers 630. These ratios place both species in Cruden's (1977) range for facultatively outcrossed species, which agrees with the results of the breeding system experiments. Control flowers of *N. lutea* in Norway and Germany and of *N. pumila* in Norway had a significantly higher seed set than either emasculated flowers, bagged and then experimentally outcrossed flowers, or emasculated and naturally outcrossed flowers. Thus, physical damage incurred during emasculation and bagging as well as the loss of visual and olfactory attractiveness of emasculated flowers to insects negatively affected seed set.

Floral development and insect visitation. First day (female stage) flowers of *N. lutea* remained almost completely closed except for a small triangular opening just above the stigmatic disk. The only way for an insect to enter these flowers and reach the nectar was to land on, or crawl over, the stigma, which during this floral stage was covered with copious sticky mucus. The mucus was devoid of glucose. The flowers emitted an intense sweet odor, and minute drops of nectar (positive reaction with diabetes test paper) were visible on the petal nectaries. In both species, stigmatic rays of first-day flowers and newly dehisced anthers stained strongly with neutral red, while nectaries did not stain, implying that terpenoid and/or lipophilic substances, which often are associated with floral scents, were present in the anthers and stigma rays, but not the nectaries. The intensity of the reaction weakened with flower age, and three day old flowers showed almost no reaction with neutral red. No visible changes in the position of sepals and petals occurred during the night. The second morning the flowers opened widely, becoming bowl-shaped. At least one row of stamens bent backwards, presenting pollen, while the mucus on the stigmatic rays dried out gradually. Nectaries looked dry on the second day, and the floral odor became somewhat pungent, but less intense than it had been the first day. During hot weather (air temperature above 30 °C) some flowers opened more widely than usual, and in the evening, the sepals of these flowers would curve until the flowers became bowl-shaped again. Other than that, no sepal or petal movement occurred during the four days of anthesis. During each of the following three days, one to two rows of anthers matured and presented pollen, while the now completely dry stigma gradually changed colour from dark yellow to brown and then green. Following the maturation of the last row of anthers, the petals and stamens withered, the peduncles lost their rigidity, and fruit ripening proceeded.

Floral development in *N. pumila* was generally the same except for the total closure of the flowers in the afternoon at around 4 p.m.; flowers opened again the next morning at 8 a.m. Male stage flowers of *N. pumila* did not open as widely as those of *N. lutea* even during hot days, nor was there a visible change in the amount of nectar presented in second-day flowers. To the human nose, the floral odor was less intense than in *N. lutea.*

Flowers of *N. lutea* were visited by 12 species of flies, bees and beetles in Germany and by 17 in Norway, while *N. pumila* was visited by four species of flies and beetles (Table 2); however, *N. pumila* was observed for only half as many hours as was each of the populations of *N. lutea.* The majority of visitors to both species were flies (e.g., 70% of the 327 recorded visits to *N. lutea* in Germany were by flies). Especially abundant among these flies were the scatophagid *Hydromyza livens* (the most frequent visitor at the German study site), the ephydrids *Hydrellia griseola* and *Notiphila brunnipes* (the most frequent visitor at the Norwegian site), the syrphid *Episyrphus balteatus*, and the muscid *Thricops cunctans. Episyrphus balteatus* was seen only on old male stage flowers where it fed on pollen.

Different from *Episyrphus*, the nectar-foraging *Hydrellia*, *Notiphila*, *Hydromyza*, and *Thricops* visited all floral stages, touching both anthers and receptive stigmas. The behaviour of these flies differed in that *Hydrellia* would spent long periods within single flowers, while *Hydromyza* and *Thricops* visited numerous flowers. Flies always landed on the stigmatic disks from where they pushed their way down through the stamens to the nectaries at the back of the petals. Most were visibly covered with pollen, especially on their feet. Often, a fly would also sit on a petal, head down, in order to reach a nectary with its proboscis; resting and cleaning took place on the stigmatic disk. Because of its abundance, large size relative to the flowers, and frequent inter-floral flights, *Hydromyza livens* may have been the most effective pollinator of *N. lutea* at our study sites (Table 2), and it also regulary

Table 2. Pollen-carrying insects collected on flowers of *Nuphar lutea* in Germany and Norway, and of *N. pumila* in Norway. Effective pollinators are marked with an *; see text for details of insect behavior

	N. lutea Germany	*N. lutea* Norway[a]	*N. pumila* Norway
Beetles			
Anaspidae			
Anaspis frontalis (L., 1758)	–	+	–
Chrysomelidae			
Donacia crassipes Fab., 1775	–	+	+
Gallerucella nymphaeae (L., 1758)	–	+	–
Nitidulidae			
Meligethes aeneus (Fab., 1775)	+	–	–
Flies			
Ephydridae			
Hydrellia griseola Fallén, 1824	+*	–	+*
Notiphila brunnipes (Rob.-Des.,1830)	–	+*	–
Anthomyiidae			
Fannia subpellucens (Zetterstedt, 1845)	–	–	+*
Muscidae			
Limnophora riparia (Fallén, 1824)	–	+*	–
Thricops cunctans (Meigen, 1826)	–	+*	+*
Scatophagidae			
Hydromyza livens (Fab., 1794)	+*	+*	–
Syrphidae			
Eoseristalis oestraceus (L., 1758) (= *Eristalis oestracea* L., 1758)	–	+	–
Episyrphus balteatus (De Geer, 1776)	+*	+*	–
Fagosyrphus cinctus (Fallén, 1817)	–	+	–
Ferdinandea cuprea (Scop., 1763)	+	–	–
Metasyrphus corollae (Fab., 1775)	+	–	–
Scaeva pyrastri (L., 1758)	+	+	–
Sphaerophoria sp.	+	–	–
Temnostoma vespiforme (L., 1758)	–	+	–
Tachinidae			
Eriothrix rufomaculata (De Geer, 1776)	–	+	–
Wasps			
Paravespula rufa (L., 1758)	+*	+*	–
Bees			
Apis mellifera L., 1758	+*	+*	–
Bombus pratorum L., 1761	–	+*	–
Bombus terrestris L., 1758	+*	+*	–
Halictus sp.	+*	–	–

[a] from Ervik & al. (1995)

pollinates *N. lutea* in the Netherlands (BROCK & VAN DER VELDE 1983). Indeed, the common name of *Hydromyza livens* in German is "Mummelfliege" or yellow water lily fly. *Hydromyza* not only forages on floral resources, but also preys on *Nuphar*-visiting *Hydrellia* and on *Nötiphila* eggs (see also BROCK & VAN DER VELDE 1983; ERVIK & al. 1995).

Numerically less important pollinators of *N. lutea* were *Apis mellifera* (12% of all visits at the German site), *Bombus terrestris* (5%), a species of halictid bees, and *Paravespula rufa* (6% of all visits). Older flowers were more atractive to pollen-seeking bee individuals, younger flowers to nectar-seeking ones. *Nuphar pumila* was not visited by bees (Table 2).

Of the three species of flies pollinating *N. pumila* in Norway, only one (*Thricops cuctans*) also pollinated *N. lutea* nearby, while another (*Hydrellia griseola*) also pollinated *N. lutea* in Germany (Table 2). Exclusively found on *N. pumila* was *Fannia subpellucens* (*Anthomyiidae*), a relatively large fly foraging for nectar and therefore visiting numerous flowers. In *N. pumila*, different from *N. lutea*, second-day flowers continue to offer nectar. Still, all flies showed a weak preference for older, male stage flowers, possibly because first-day, female stage flowers in *N. pumila* have only a small apical opening and are difficult to crawl into.

Of the ten species of flies visiting *N. lutea* in Norway (Table 2), only *Thricops cunctans* also visited, and pollinated, nearby *N. pumila.*

Beetles found on flowers of *N. lutea* in Germany and Norway were *Anaspidae*, *Chrysomelidae* and *Nitidulidae* (Table 2), too small and stationary to effect cross-pollination, but contributing to insect-mediated selfing. The larger chrysomelid *Donacia crassipes* was found on old male stage flowers at the Norwegian site, where it was also present on leaves, but not at our German site. On *N. pumila*, three individuals of *D. crassipes* were found inside second-day and third-day (male stage) flowers, and one was found feeding on the sepals of a flower. One of them had *Nuphar* pollen attached to its abdomen. *Donacia sparganii* AHRENS, a specialist on *Sparganium*, was abundant at some of the Norwegian sites and was occasionally seen on *Nuphar* leaves, but never inside flowers.

Discussion

This study shows that *N. lutea* and *N. pumila* are dependent on insects for seed production and that the most frequent visitors to both species are flies, some of which effectively pollinate the flowers. Honey bees and bumble bees play a role as pollinators in *N. lutea* but not *N. pumila.* Both species are selfcompatible and have protogynous flowers, with the only overlap between the female and male stages occurring during the morning of the second day when the stigmatic rays are still humid and receptive. At that time, insect-mediated geitonogamy may occur, but seed set in experimentally pollinated second-day flowers was significantly less than in first-day flowers. SCHNEIDER & MOORE (1977) reported that bagged flowers of *N. lutea* subsp. *macrophylla* (= *N. advena*) in Texas produced some seeds by automatic selfing. The mechanism for this is unclear.

In agreement with two earlier studies of European *Nuphar* (VAN DER VELDE & al. 1978, ERVIK & al. 1995) and with ROBERTSON's (1889) observations in Illinois and Florida, and contrary to coevolutionary scenarios linking *Nuphar* pollination to *Donacia* (SCHNEIDER & MOORE 1977, SCHNEIDER 1979, SCHNEIDER & WILLIAMSON

1993), we found that *Nuphar* does not rely on *Donacia*, or other, beetles for cross pollination.

Mouthparts of species of *Donacia* associated with *Nuphar* and *Nymphaea* in the northern hemisphere are specialized for leaf feeding and are unsuited for eating pollen or taking up nectar (ASKEVOLD 1988, 1990, 1991). Mating in these beetles does not take place in flowers either; rather, it takes place under water, and eggs are then laid on the lower surface of *Nuphar* or *Nymphaea* leaves depending on the particular species of *Donacia*. In spite of *Donacia*'s lack of adaptations for feeding on pollen or nectar, *N. advena* in Texas and *N. polysepala* in California and Washington are sometimes pollinated by species of *Donacia* in addition to honey bees and halictid bees (SCHNEIDER & MOORE 1977). This raises the possibility that particular floral features might explain differences in pollinator spectra between European and American species of *Nuphar*. One such feature might be the nocturnal closure of the flowers in *N. advena*. Closed flowers may offer a protected space for the beetles to spend the night, and this might be a precondition for the evolution of a mutualism between *Nuphar* and *Donacia*. Arguing against this interpretation, however, is the fact that there is no clear correlation between flower closure and visitation by *Donacia*. British populations of *N. lutea*, which, different from continental populations, close their flowers (HESLOP-HARRISON 1955), still are visited and pollinated by flies and bees, rather than by *Donacia*, and the same is true of *N. pumila* in Norway, which also closes its flowers (see Results). Also, neither *Nymphaea odorata* nor *N. alba*, species that commonly co-occur with *Nuphar* and whose flowers close overnight, are used for shelter by *Donacia* species that feed on their leaves. Instead, early in the day before air temperatures rise, *Donacia* are found on leaves or stems under water (ASKEVOLD 1988), which may indicate that they seek shelter there rather than in flowers. Independent of selection by flower-visiting beetles, floral closure may help protect pollen and nectar from humidity, or conversely prevent the drying out of stigmatic mucus and nectar. Protection from drying out would explain why nectar is still available in second-day *N. pumila* flowers, which close overnight, but not in *N. lutea* flowers, which do not close.

Other floral features of *Nuphar* are common to American and European species and do not suggest adaptation to beetle pollinators. Thus, the dorsobasal petal nectaries can be exploited by short-tongued nectar-seeking flies and bees, and the sweet floral scent and ultra-voilet pattern (GIESEN & VAN DER VELDE 1983, LIPPOK 1995) created by the different reflectance behaviour of the sepals, anthers and stigmatic disk also fit with fly and bee pollination.

A cladistic analysis based on floral characters of all eight nymphaealean genera by WILLIAMSON & SCHNEIDER (1994) placed *Nuphar* as either a sister clade to *Euryale*, *Nymphaea*, and *Victoria* or as basal to the *Barclaya-Ondinea* clade, and a cladogram by LES & al. (1991) using rbcL sequence data and including seven of the eight genera included by WILLIAMSON & SCHNEIDER (1994) shows a similarly unresolved position of *Nuphar*. Therefore, phylogenetic information at present is insufficient to hypothesize on ancestral floral features in *Nuphar* based on sistergroup or outgroup comparisons. However, the empirical observations on the pollination of European species of *Nuphar* discussed here and a comparison of their floral features with those of the more numerous American species suggest that ancestors likely had fly- and bee-pollinated flowers. We base this hypothesis mainly on the apparently synapomorphic dorsobasal petal nectaries and the yellow flowers.

Initially we asked whether allopatric populations of single species of *Nuphar* are pollinated by different pollinators while sympatric populations of different species are pollinated by the same pollinators as would be expected for open flowers with seemingly easily accessible rewards. It turned out that sympatric populations of *N. pumila* and *N. lutea* are indeed pollinated by some of the same species of flies. At the same time, their pollinator spectra differed strikingly. *Nuphar lutea*, but not *N. pumila*, was visited by *Apis*, *Bombus*, and *Paravespula*, even though these species were present in the both habitats, and only *N. lutea*, but not *N. pumila*, was visited by syrphids. The five syrphid species involved are common throughout Scandinavia and known to visit numerous bog and swamp flowers (J. M. OLESEN, pers. comm.). Possibly, bees, wasps, and syrphids prefer *N. lutea* over *N. pumila* because of its larger, more open flowers, which offer more pollen and nectar. In contrast to these visitors, the scatophagid fly *Hydromyza livens* is a *N. lutea* specialist that probably visits *N. lutea* flowers throughout Europe. Also some ephydrid flies, especially *Notiphila*, appear to be regularly associated with *Nuphar*; ROBERTSON (1889) found an unidentifed species of *Notiphila* pollinating *N. advena* in Florida, and we found *Notiphila brunnipes* pollinating *N. lutea* in Norway. Besides these apparent *Nuphar* specialists, populations of *N. lutea* in Norway and Germany share about half their other fly and bee pollinators. Additional studies of *Nuphar* pollination at other sites, especially in North America, would allow us to further assess the degree to which the different species may rely on different groups of insects for pollination.

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