NUPHAR INTERMEDIA LEDEB., A PRESUMED RELICT HYBRID, IN BRITAIN

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INTRODUCTION

Nuphar intermedia was originally described by Ledebour (1832) from plants obtained from Heiligenzee and Zee von Camby in North Germany. As was implied by the name given it, the species was intended to embrace plants which lay between the generally accepted variation ranges of *N. lutea* (L.) Sm. and *N. pumila* (Timm) DC. Subsequently, Caspary (1870) and other students of the Nymphaeaceae pointed out the similarity between wild plants which had been referred to Ledebour's *N. intermedia* and artificial hybrids

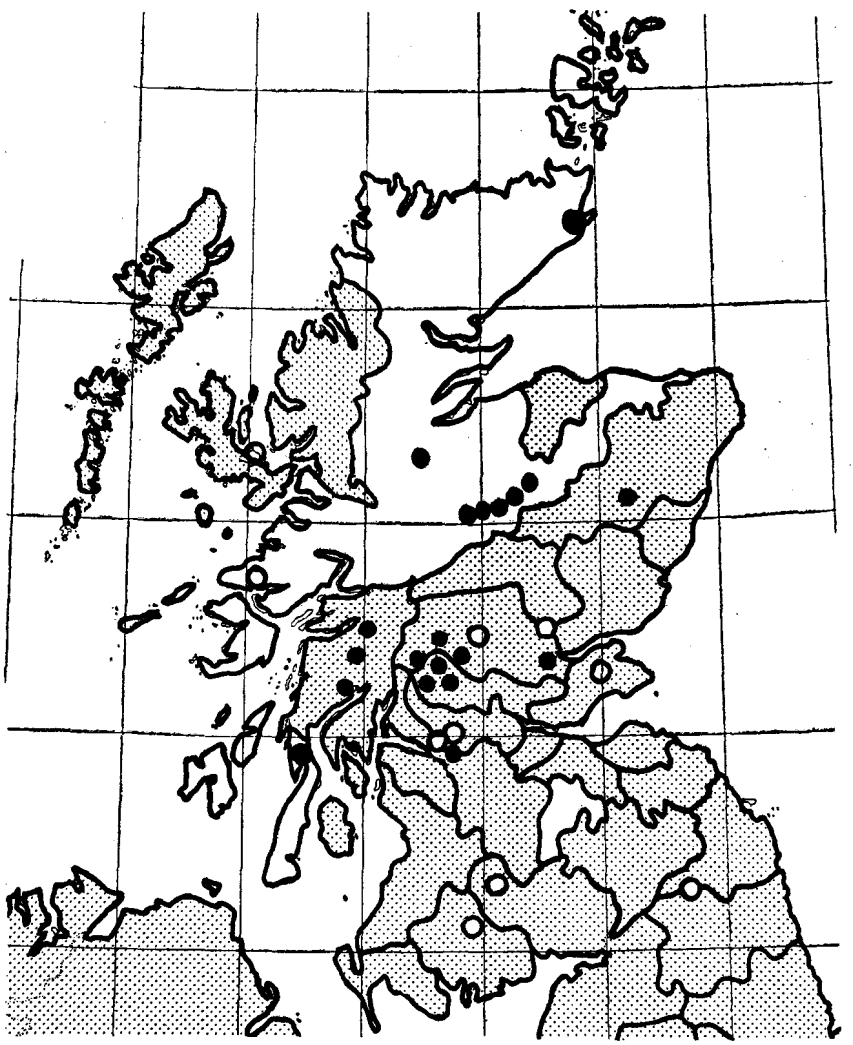

Fig. 1. Scottish and northern English ranges of *N. intermedia* and its presumed parents *N. pumila* and *N. lutea*. *N. intermedia* occurs at *open circles*, *N. pumila* at *black circles* and *N. lutea* in *shaded vice-counties*.

between *N. lutea* and *N. pumila*, and it has since been customary to assume that *N. intermedia* does not in fact represent a pure breeding species but simply an assemblage of natural hybrids. Particular interest attaches to this ascription of a hybrid origin to *N. intermedia* because, in many localities from which this taxon has been reported, one or other, and sometimes both, of the presumed parents are missing. That this is so for certain British stations of *N. intermedia* is apparent from Fig. 1, which shows the Scottish and northern English ranges of the two parents and the presumed hybrid. The distribution of *N. lutea* is shown by vice-counties, those of *N. pumila* and *N. intermedia* by spot marking, the localities indicated being those from which specimens exist in the herbaria of the British Museum (Natural History), the Royal Botanic Gardens of Kew and Edinburgh, and Cambridge University. *N. lutea* is, of course, mainly a lowland plant of lakes, slow rivers and canals, while *N. pumila* today is, for the most part, confined to high-level lakes in mountainous regions, an exception being the remarkable colony which exists in one of the Shropshire meres, a locality first reported by Cox (1855). While many of the colonies of *N. intermedia* occur within the area of overlap of the two presumed parents, one, in Ardnamurchan, is in a vice-county (v.c. 97, Westerness) from which neither has been recorded; and others, in southern Scotland and in Northumberland, are remote from areas where *N. pumila* is known today.

If *N. intermedia* is indeed a hybrid, this persistence outside of the range of its presumed parents is of considerable historic as well as taxonomic interest. The present paper contains a review of the evidence for accepting it as such, as well as some speculation relating to its possible origin in the British stations. Particular attention is paid to the colony in Chartners Lough, Northumberland, where *N. intermedia* exists in a station very distant from the nearest " pure " *N. pumila*. The important work of Caspary in connection with the problem of *N. intermedia* in Continental Europe is also reviewed.

N. intermedia in Chartners Lough, Northumberland

This station for *N. intermedia* was first discovered by Sir John Trevelyan, and placed on record by Winch (1832), in his remarkable flora of Northumberland and Durham, as *N. lutea* var. *β pumila*. From this date Chartners became a classical locality for the plant, featuring in practically all local and national floras in which the genus was treated in any detail. The taxonomic treatments given it differed according to the nomenclature currently in favour :

N. J. Winch (1832),* " *N. lutea* var. *β pumila* " (collector Sir J. Trevelyan).
H. C. Watson (1835), " *N. pumila* " (collector Sir W. C. Trevelyan).
W. J. Hooker (1835), " *N. pumila* (= *N. kalmiana*) " (collector Sir J. Trevelyan).
H. C. Watson (1847), " *N. pumila* De C." (collector Sir W. C. Trevelyan)
W. J. Hooker & G. A. W. Arnott (1855), " *N. pumila* De C."
J. T. Syme (1863), " *N. lutea* Sm. var. *β minor*."
J. G. Baker & G. R. Tate (1868), " *N. intermedium* Ledeb."
J. D. Hooker (1884), " *N. luteum* Sm. var. *N. intermedium* Ledeb."
W. R. Hayward (1892), " *N. luteum β N. intermedia* Ledeb."
J. L. Luckley (1893), " *N. lutea* var. *β*."
C. E. Moss (1920), " *N. pumila* var. *intermedia* Moss."

Winch himself (1832) reported the results of what must be one of the earliest examples of British experimental taxonomy – a transplant experiment on the Chartners plant – in the following words : " Some years since, the least yellow water lily was transplanted from the subalpine moors into the ponds at Wallington, where it now scarcely differs

* Referred to as *N. minima* in a letter dated 26.4.24 from Winch to J. E. Smith (Smith 1832).

from the common water lily; in both varieties, at least I consider them as such, the stigma is entire when young, but becomes toothed as it grows older." H. C. Watson (1847) commented upon this report as follows: "A specimen in my herbarium, from Sir W. C. Trevelyan, appears to confirm this transformation, but Mr. Borrer has lately explained that the Northumberland plant is not *pumila*, but a rather small variety of *N. lutea*. In size of flower and leaf the specimen corresponds more closely with the Highland *N. pumila*; while in the repand margin, the specimen is certainly more like *N. lutea*."

Baker and Tate (1868), whose flora of Northumberland and Durham superseded that of Winch, were also familiar with the Chartners Lough plant and the Wallington transplants. They considered that the plant agreed with the *N. intermedia* of Ledebour, but were led by the absence of *N. pumila* from the vicinity to dismiss the possibility that it could have arisen from the hybridisation of *N. lutea* and *N. pumila*.

Luckley (1893) gave a final report on Sir J. Trevelyan's transplant experiment, contradicting Winch in declaring that the Chartners plant " discovered about 100 years ago by Sir J. Trevelyan, was introduced into a pond at Wallington, where I saw it growing during the lifetime of the late Sir W. C. Trevelyan Bart., without the plant having undergone any change."

The Chartners plant has been assiduously collected by generations of British botanists, and herbarium sheets of it exist in most of the larger herbaria. Three sheets of particular interest are those of material collected by W. C. Trevelyan, the two incorporated in the herbaria of Winch and H. C. Watson, whose comments are quoted above, and another bearing Caspary's signature, dated 1856, declaring the specimen to be *N. kalmiana* of Hooker's " Flora Scotica," or *N. minima* of Syme's " English Botany " (1825). Other interesting sheets are those of material collected by Fraser Robinson in 1905. On one of these, in the British Museum, there is a note which states that *N. intermedia* was the only water lily present in Chartners Lough, where it was " still very abundant," and that the lough itself appeared to show signs of drying out, being about half an acre in extent.

Chartners Lough lies on the Wallington Moors at a height of c. 1,050 ft., about a quarter of a mile from the isolated farm of Chartners. The appearance of the lough today clearly differs little from that when Fraser Robinson's note was written, and the area of open water is still about half an acre. The east bank is overhung by peat hags and evidently erosion is actively in progress here, since the peat is severely undercut. It seems that Chartners, like other small loughs in the neighbourhood, may actually be migrating slowly eastwards (Smythe, 1930). On the west lies a small area of bog containing *Sphagnum recurvum* P. Beauv., *Oxycoccus palustris* Pers., *Polytrichum commune* Hedw., *Carex rostrata* Stokes and *Eriophorum vaginatum* L. At the margin of the lough there is a narrow zone of reedswamp with *Carex rostrata* and *Juncus effusus* L., and *Sphagnum cuspidatum* Hoffm. grows in the water. The greater part of the water surface is covered by *Nuphar* plants. The lough and the neighbouring bog are enclosed by a bank of thick peat, and at a slightly lower level, between the farm and the lough itself, there is a large area of drier bog, much burnt over.

The nearest other locality for a *Nuphar* species to Chartners is at Kimmer near Eglingham, some 15 miles to the north-east, a locality mentioned by Winch for *N. lutea*. In Winch's time there were several other localities for *N. lutea* within 20 miles of Chartners, e.g. Wide Haugh near Dilston (18 miles S.S.W.), Prestwick Carr (18 miles S.S.E.) and Sewing Shields (18 miles S.W.). *N. lutea* is now extinct in many of these localities due to water pollution or drainage.

The nearest stations for *N. pumila* today are in Stirlingshire and W. Perthshire, some 85-90 miles to the north-west. *N. intermedia* is recorded in Dumfriesshire and Lanarkshire, some 65 miles to the west.

MORPHOLOGICAL COMPARISONS BETWEEN *N. intermedia*, *N. lutea* AND *N. pumila*

For the purpose of a morphological comparison of *N. intermedia* with its presumed parents a random collection of 30 flowers has been taken from the Chartners colony. This has been compared with samples of *N. lutea* from the following twelve localities (L. 1-12):

1. Royal Canal, nr. Ballycormack, Co. Longford, v.c. H 24.
2. Loch Bannow, Lanesborough, Co. Longford, v.c. H 24.
3. Lough Craiggamore, W. Galway, v.c. H 16.
4. R. Thames at Cookham, Berkshire, v.c. 22.
5. Lode near Wicken Fen, Cambridgeshire, v.c. 29.
6. Lode near R. Ant, Sutton Broad, E. Norfolk, v.c. 27.
7. Ellesmere, Shropshire, v.c. 40.
8. Small lake in Tregaron Bog, Cardiganshire, v.c. 46.
9. Esthwaite Water, N. Lancashire, v.c. 69.
10. Loughrigg Tarn, Westmorland, v.c. 69.
11. Grasmere, Westmorland, v.c. 69.
12. Monkhill Lough, near Carlisle, Cumberland, v.c. 70.

and of *N. pumila* from Lochanovie, Easterness, v.c. 96 (P 1), and Shropshire, v.c. 40 (P 2).

In Table 1, in which the biometrical data for the three taxa are compared, the *N. lutea* samples are aggregated so that the information given may be taken to be representative of a general lowland sample of this species. The data for the *N. pumila* samples are given separately for the floral characters (except for petal length) so that comparison may be made between the population from Lochanovie and the interesting isolated Shropshire colony, both of which are regarded as being typical *N. pumila*. The Avinlochan colony (N. of Aviemore, Inverness, v.c. 95—P 3), discussed further below, is possibly itself affected by hybridisation, and the data for it have not been included in the *N. pumila* aggregate.

TABLE 1

Meristic and size data for floral characters in British *Nuphar* taxa. For localities see text. (All size measurements in mm.)

Taxon sample	Sepal		Petal		Carpel	
	length	width	number	length	number	diameter
N. pumila agg. (P1 + P2)	20·13 ± 0·24	12·54 ± 0·20	11·42 ± 0·17	4·74 ± 0·11	9·42 ± 0·11	7·96 ± 0·14
P1	20·77 ± 0·36	13·20 ± 0·22	11·63 ± 0·21		9·26 ± 0·15	7·88 ± 0·19
P2	19·56 ± 0·31	12·14 ± 0·33	10·86 ± 0·13		9·54 ± 0·16	8·36 ± 0·15
P3(Avinlochan)	23·54 ± 0·44	13·45 ± 0·43	10·56 ± 0·41	5·00 ± 0·13	10·90 ± 0·20	10·11 ± 0·23
N. intermedia	25·23 ± 0·59	19·80 ± 0·46	9·82 ± 0·25	8·22 ± 0·34	11·58 ± 0·18	9·20 ± 0·18
N. lutea agg. (L1 — 12)	33·76 ± 0·77	31·70 ± 1·14	15·11 ± 0·22	15·12 ± 0·25	15·78 ± 0·19	12·40 ± 0·22

FLORAL CHARACTERS

Both meristic and size characters have been compared, the dimensions selected in the latter case being those which are most readily and consistently assessable, viz. maximum length and width of largest sepal, length of largest petal and diameter of stigmatic disc. The meristic characters are petal and carpel number.

(1) *Sepals.* The sample data of Table 1 for dimensions of largest sepal show that the two *N. pumila* samples hardly differ significantly for this character ($p \simeq \cdot 04$). The Avinlochan population evidently has somewhat longer sepals, while in the Chartners population of *N. intermedia*, the average sepal length is some 25 per cent greater and the average sepal width about 50 per cent greater than in *N. pumila*. Clearly these figures also indicate a shape difference, which is illustrated in Fig. 2. The sepals of *N. lutea* are rounded (Fig. 2a), those of *N. pumila* obovate (Fig. 2d) and those of *N. intermedia* intermediate in shape as well as in size (Fig. 2b). Characteristically all three possess five sepals. There is, however, some variation in this feature, as can be seen from Table 2.

TABLE 2

Distribution of sepal number in British *Nuphar* taxa. Percentage representation in each class.

Taxon	No. of sepals			
	4	5	6	7
N. pumila (P1 + P2)	3·46	93·10	1·72	1·72
N. intermedia (Chartners)	—	89·18	—	10·82
N. lutea (L1 — 12)	—	95·73	4·27	—

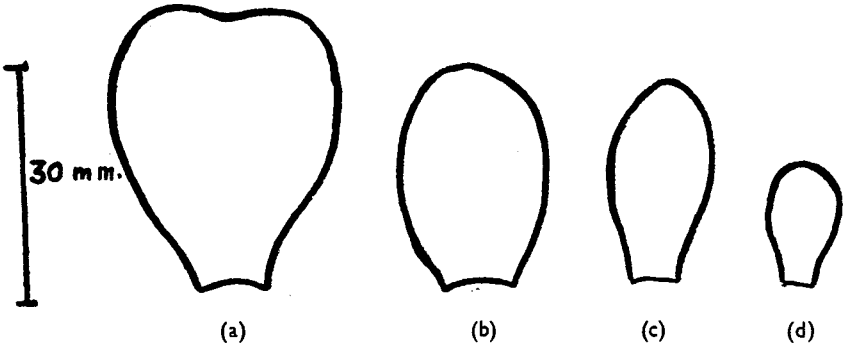

Fig. 2. Characteristic sepal shapes in British *Nuphar* taxa. (a) *N. lutea* from Ellesmere; (b) *N. intermedia* from Chartners Lough; (c) " introgressed " *N. pumila* from Avinlochan and (d) *N. pumila* from Lochanovie.

(2) *Petals.* The size relationship apparent between the three taxa in sepal length holds also in petal length, as may be seen from the data for this character given in Table 1, except that proportionately the petals of *N. intermedia* exceed those of *N. pumila* to a greater extent. Characteristic shapes are illustrated in Fig. 3.

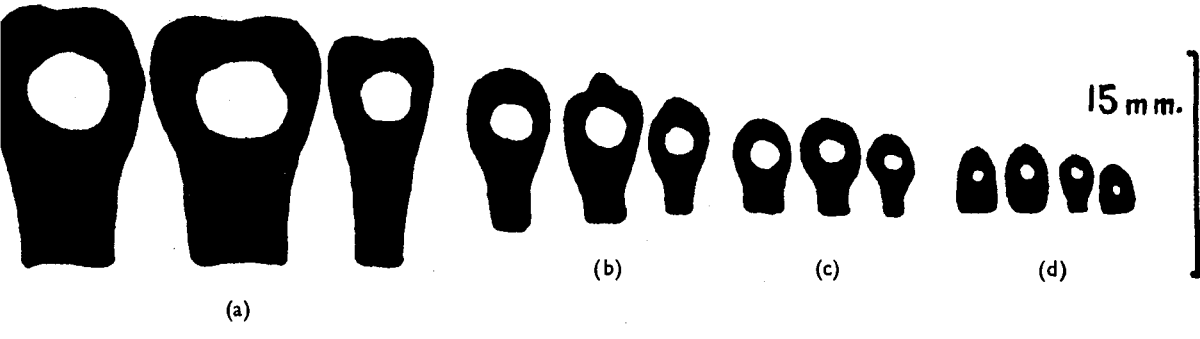

Fig. 3. Characteristic petal shapes in British *Nuphar* taxa. Circles mark the position of the nectaries. (a) *N. lutea*; (b) *N. intermedia*; (c) " introgressed " *N. pumila*; and (d) *N. pumila*. Sources of material as in Fig. 2.

The sample data of Table 1 for petal *number* would seem to suggest that the three taxa stand in a different relationship, since the mean number of petals for *N. intermedia* is, statistically speaking, significantly less than in the *N. pumila* aggregate. However, as will be seen from the data of Caspary given in Table 6, in certain populations of continental *N. intermedia* the average petal number may actually exceed the average number found in British *N. lutea*, a matter discussed further below.

(3) *Stamens.* The variation in stamen shape has been observed by several authors, e.g. Syme (1863) and Moss (1920). Thus, according to Syme, in *N. pumila* the anthers are not more than one and a half times as long as broad. Koch (1843) stated that this feature, among others, differentiates *N. pumila* from *N. spenneriana* Gaudin, in which the anthers are said to be four times as long as broad. Planchon (1853), however, believed

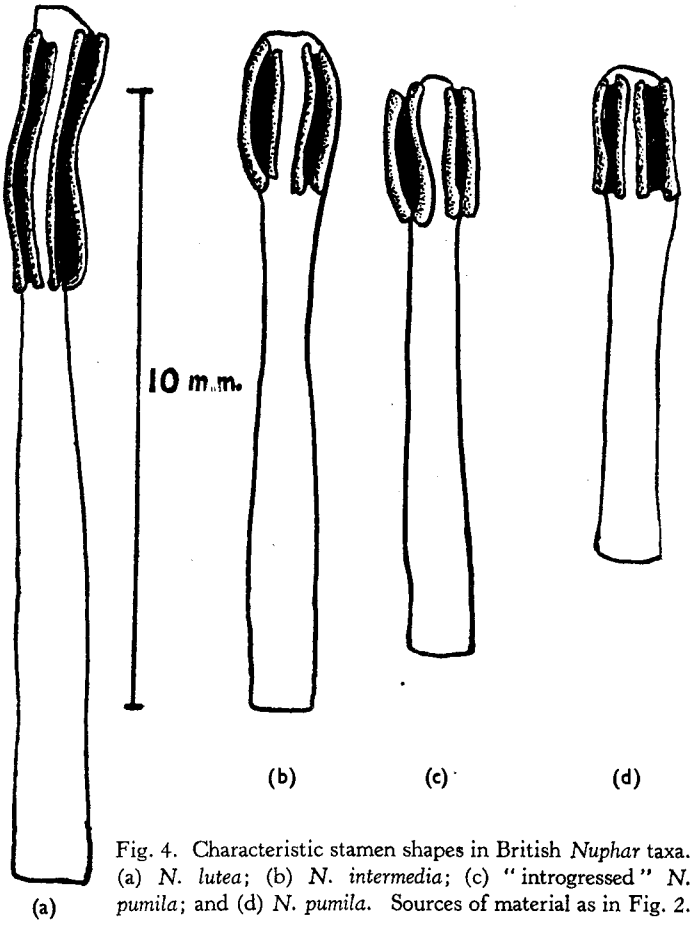

Fig. 4. Characteristic stamen shapes in British *Nuphar* taxa. (a) *N. lutea*; (b) *N. intermedia*; (c) "introgressed" *N. pumila*; and (d) *N. pumila*. Sources of material as in Fig. 2.

the character of anther length to be variable in *N. pumila*, ranging from oblong (four times as long as broad) to almost square. Planchon noted that he had seen specimens described as *N. pumila* from Scotland and America with elongated anthers. Moss (1920) used anther shape as a discriminating character in dealing with *N. lutea* and *N. pumila*, stating that the anthers in the former were usually four times as long as broad, and in the latter about twice as long as broad.

In the *N. pumila* from Lochanovie and Shropshire the anthers have a squarish form (Fig. 4d) whereas those of *N. lutea* in all the British populations examined are considerably elongated (Fig. 4a). Again *N. intermedia* is intermediate in this character (Fig. 4b). The

anther shape in the Avinlochan colony (Fig. 4c) is somewhat elongated compared with normal *N. pumila*, approaching *N. intermedia* in this character. It is possible that the Scottish plants with elongated anthers seen by Planchon came from this locality.

Statistical data for stamen *number* have not been obtained from all the *Nuphar* colonies investigated, but the counts that have been made suggest a mean stamen number for *N. lutea* of c. 105, for *N. intermedia* of c. 66 and for *N. pumila* of c. 52.

(4) *Pistils.* In the dimensions of the stigmatic disc the size relationships of the three taxa parallel those in sepal and petal length, with *N. intermedia* again intermediate between *N. lutea* and *N. pumila*. The Avinlochan colony is quite anomalous in this character; the mean value given in Table 1 refers to maximum diameter and this is high, owing to a certain amount of ovary fasciation found in many individuals of this colony (Fig. 6e).

Carpel *number* is an important diagnostic in *Nuphar*. The two *N. pumila* samples, P 1 and P 2, are in close agreement in this character, and the *N. intermedia* is again intermediate between these and the *N. lutea* aggregate sample. The relationships of the three are shown graphically in Fig. 5. As a result of fasciation in individuals of the Avinlochan sample (see Fig. 6e), the mean carpel number is greater here than in the other two *N. pumila* samples. The frequency distribution of carpel number in this sample is compared with that in the *N. intermedia* and *N. pumila* samples in Fig. 7.

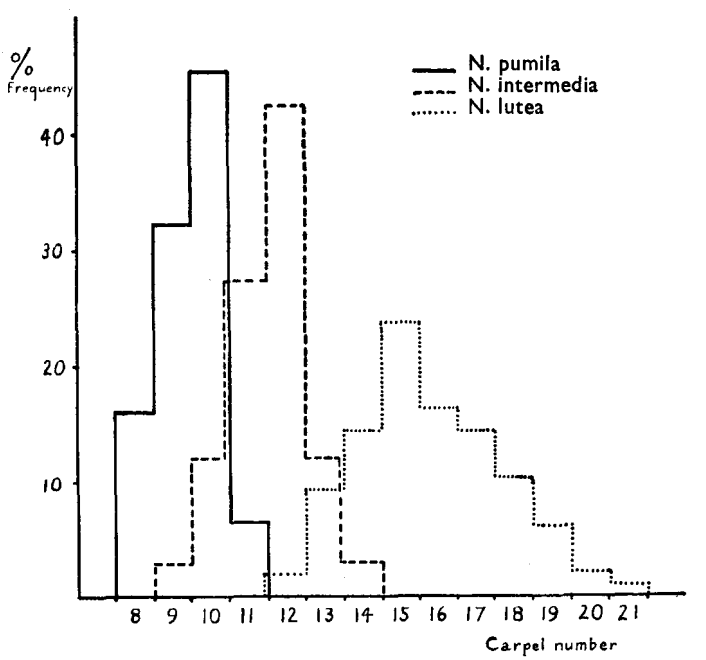

Fig. 5. Frequency histograms of carpel number in British *Nuphar* taxa.

Particularly in *N. lutea*, the stigmatic rays are singularly variable in form. This feature seems to have been overlooked previously by European taxonomists, although in N. America, where the genus is highly polymorphic, Miller and Standley (1912) have emphasized its importance in the diagnoses of some seventeen species. The stigmatic discs illustrated in Fig. 6a-c give some indication of the variability in this character in *N. lutea*; the individuals from which drawings were made were derived from Tregaron and Ellesmere. The rays may be linear and widely spaced, ovate, or so broad as to

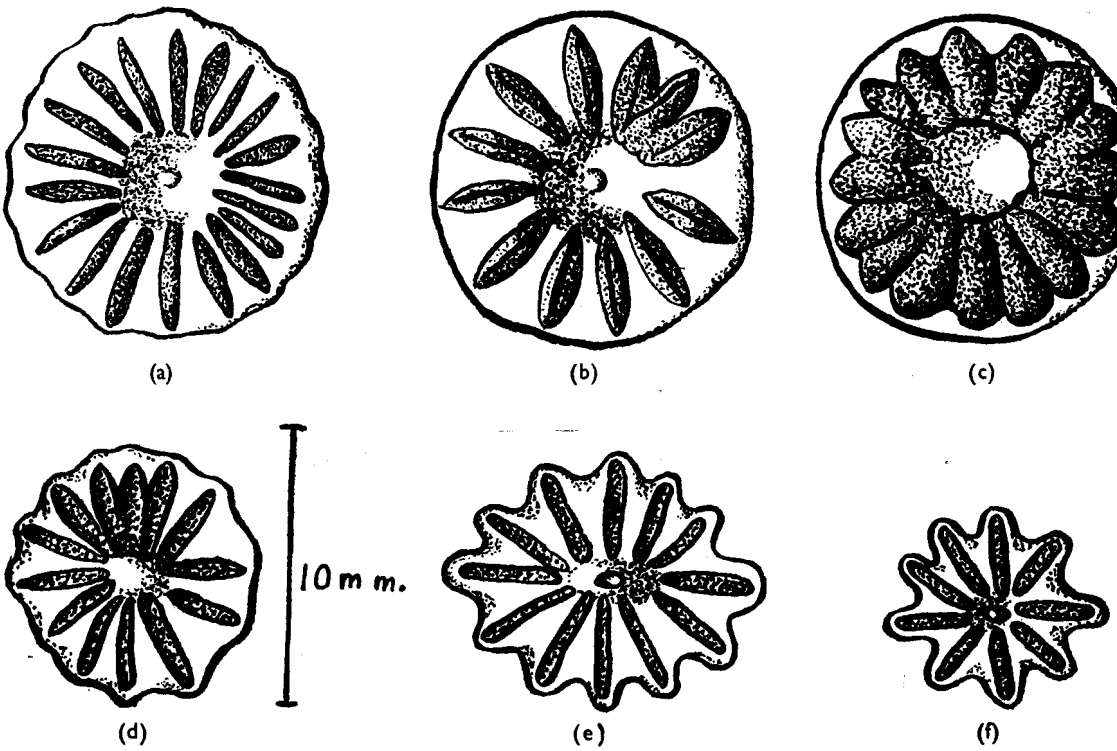

Fig. 6. Characteristic forms of stigmatic disc in British *Nuphar* taxa. (a)-(c) *N. lutea*; (d) *N. intermedia*; (e) " introgressed " *N. pumila*; and (f) *N. pumila*. *N. lutea* from Ellesmere and Tregaron, other sources of material as in Fig. 2.

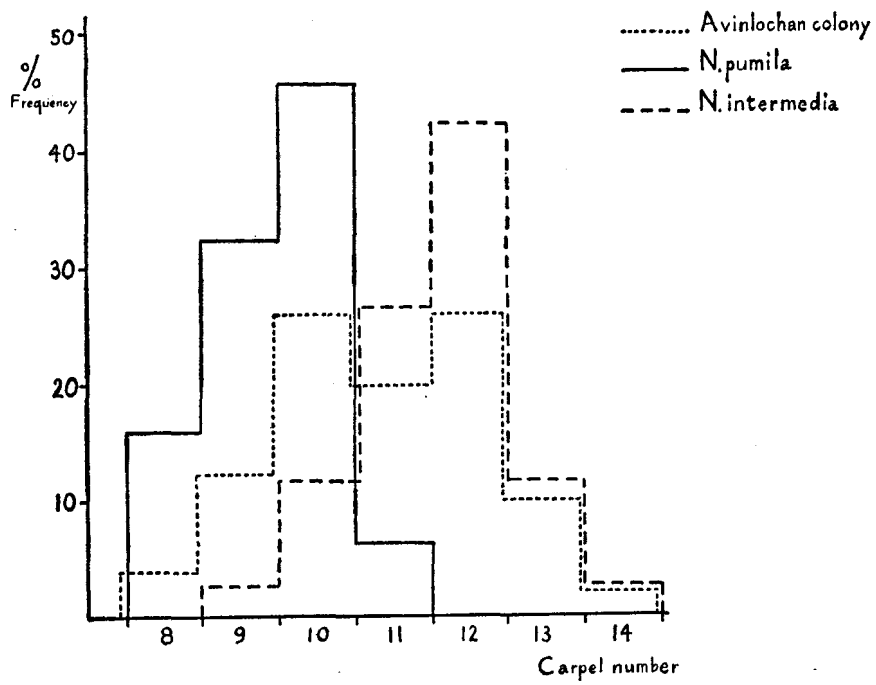

Fig. 7. Frequency histograms of " introgressed " *N. pumila*, *N. pumila* and *N. intermedia*.

coalesce and present a continuous ring of stigmatic surface on the disc. The rays themselves may be smooth or more or less furrowed, and their prominence above the surface of the stigmatic disc is likewise variable. The central depression of the disc may be shallow or deep, and with or without an umbo. The margin of the disc is characteristically entire, a key " qualitative " feature distinguishing *N. lutea* and *N. pumila*; occasionally, however, it is somewhat sinuous in *N. lutea*, as is found, for example, in some plants in the population in Sutton Broad. In Britain, at least, *N. pumila* appears to be not nearly so variable in the character of the stigmatic disc; the margin is uniformly deeply scalloped and the stigmatic rays are linear. The axile process, however, may or may not be developed.

In *N. intermedia* the disc has a distinctly undulate margin (Fig. 6d) and so is intermediate in this character between *N. lutea* and *N. pumila*. In the Chartners Lough colony the stigmatic rays, although greater in number than in *N. pumila*, are similar in

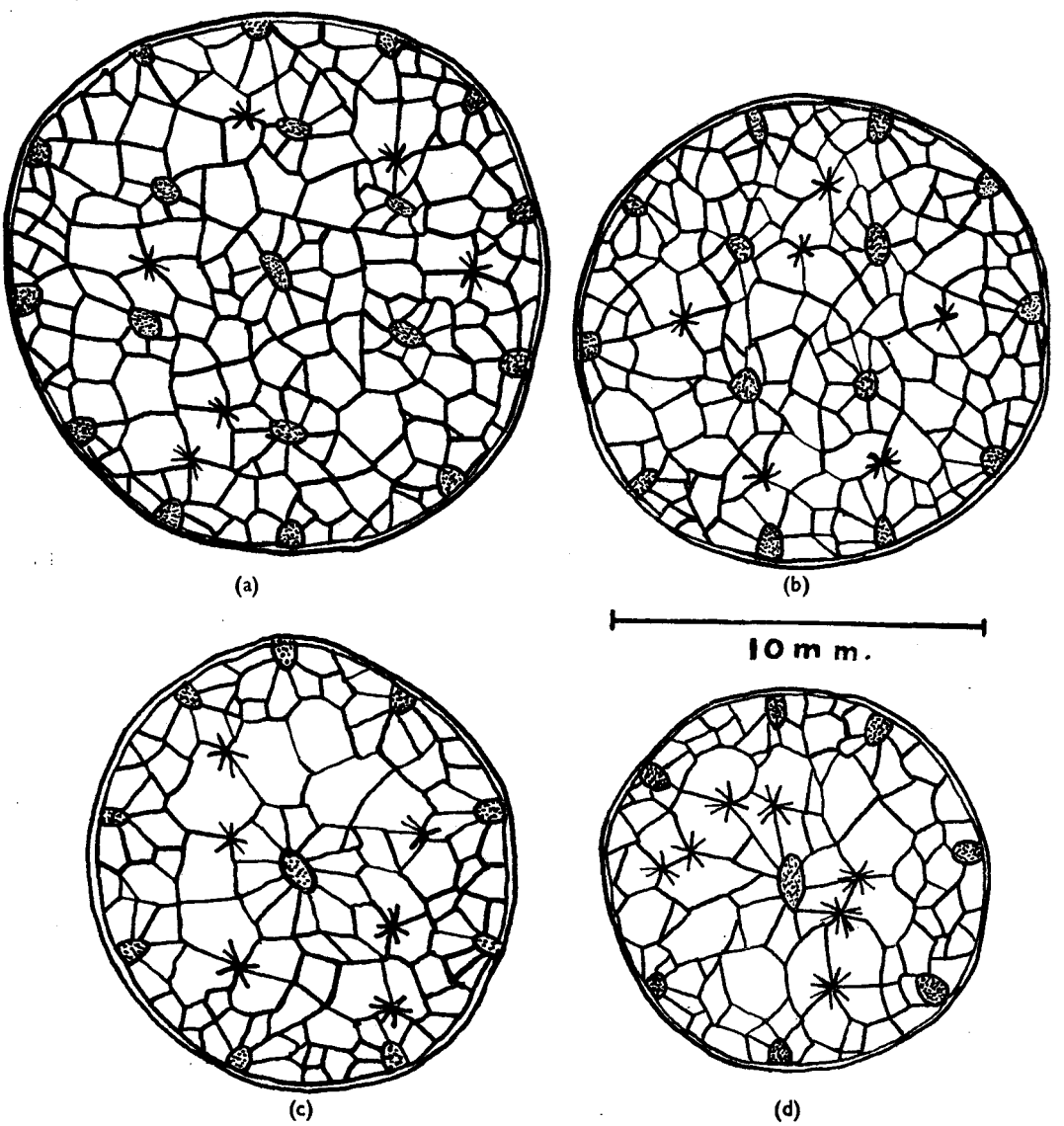

Fig. 8. Characteristic forms of peduncle anatomy in British *Nuphar* taxa. (a) *N. lutea*; (b) *N. intermedia*; (c) " introgressed " *N. pumila*; and (d) *N. pumila*. Sources of material as in Fig. 2.

form; but in some individuals there is a tendency for the rays to coalesce, as in some forms of *N. lutea*.

(5) *Peduncles*. The flower stalk in *N. lutea* is usually 50-70 per cent thicker than in *N. pumila*, and this difference in thickness is correlated with a difference in number of vascular bundles. In *N. lutea* these are usually distributed in an outer ring and an inner ring with a solitary central bundle (Fig. 8a). In *N. pumila* the central bundle and the outer ring are present, but the inner ring is missing (Fig. 8d). In the peduncles of *N. intermedia* the distribution of the bundles is much more variable, a common arrangement being an outer ring and a central group of four (Fig. 8b), so, in this character again, the condition is intermediate. In the Avinlochan colony the central bundle is retained, but there are a greater number of vascular bundles in the outer ring, compared with *N. pumila* (Fig. 8c).

Vegetative Characters

Sample data for leaf size are tabulated in Table 3, the dimensions given being maximum length and width of leaf. Again *N. intermedia* lies between *N. lutea* and *N. pumila*, approaching more closely the latter. This relationship is present also in thickness of petiole and, as with the peduncle, this is reflected in the ratio of the number of vascular bundles present in the petioles, the ranges in the plants examined being from 17-23 in *N. lutea* ($M = 19{\cdot}81 \pm 0{\cdot}41$), 8-13 in *N. intermedia*, 7-12 in the Avinlochan colony and 6-10 in *N. pumila*. The number of vascular bundles is constant from petiole base to petiole apex. There is also some difference in the shape of the petiole in cross-section, a feature mentioned in most descriptions of the species. That of *N. pumila* is elliptical or lens-shaped (Fig. 9d) and that of *N. lutea* is trigonous (Fig. 9a); again *N. intermedia* lies in between the two in this feature (Fig. 9b). The number of lateral veins in the leaf ranges from 23-28 ($M = 25{\cdot}33 \pm 0{\cdot}35$) in *N. lutea* and 11-18 in *N. pumila* (for the aggregate sample, P1 + P2, $M = 14{\cdot}50 \pm 0{\cdot}39$). In the *N. intermedia* samples studied the range was 15-22 ($M = 18{\cdot}08 \pm 0{\cdot}53$).

Table 3

Leaf characters of European *Nuphar* taxa (all size measurements in cm.).

Taxon	No. of lateral veins	Leaf	
		Length	Width
N. pumila			
P. *a*	$14{\cdot}50 \pm 0{\cdot}39$	$12{\cdot}60 \pm 0{\cdot}44$	$9{\cdot}60 \pm 0{\cdot}36$
P. *b*	$13{\cdot}86 \pm 0{\cdot}65$	$9{\cdot}87 \pm 0{\cdot}22$	$7{\cdot}38 \pm 0{\cdot}16$
P. *c*	$12{\cdot}00 \pm 0{\cdot}36$	$9{\cdot}89 \pm 0{\cdot}15$	$7{\cdot}24 \pm 0{\cdot}15$
N. intermedia			
I. *a*	$18{\cdot}08 \pm 0{\cdot}53$	$14{\cdot}80 \pm 0{\cdot}72$	$10{\cdot}90 \pm 0{\cdot}50$
I. *b*	$16{\cdot}91 \pm 0{\cdot}73$	$11{\cdot}63 \pm 0{\cdot}56$	$9{\cdot}28 \pm 0{\cdot}34$
I. *c*	$14{\cdot}33 \pm 0{\cdot}69$	$11{\cdot}10 \pm 0{\cdot}21$	$10{\cdot}09 \pm 0{\cdot}44$
N. lutea			
L. *a*	$25{\cdot}33 \pm 0{\cdot}35$	$29{\cdot}50 \pm 0{\cdot}93$	$26{\cdot}16 \pm 1{\cdot}02$
L. *b*	$22{\cdot}86 \pm 0{\cdot}64$	$23{\cdot}70 \pm 1{\cdot}46$	$18{\cdot}60 \pm 1{\cdot}23$

Sources of material : *a* – living British (for localities see text); *b* – herbarium British (vice-county sources for *N. pumila* : 40, 86-88, 92, 96-98, 109; for *N. intermedia* 68, 72, 77, 83, 85, 88, 89, 97, 109; and for *N. lutea* 6-9, 11-23, 25-39, 33, 37, 40, 41, 43, 48, 59, 70-72, 81, 86, 89, 98); *c* – herbarium continental European (sources of *N. pumila* and *N. intermedia* include Scandinavia, the Alps and neighbouring mountain systems; *N. lutea* from Scandinavia, central and western Europe, N.W. Russia and the Balkans).

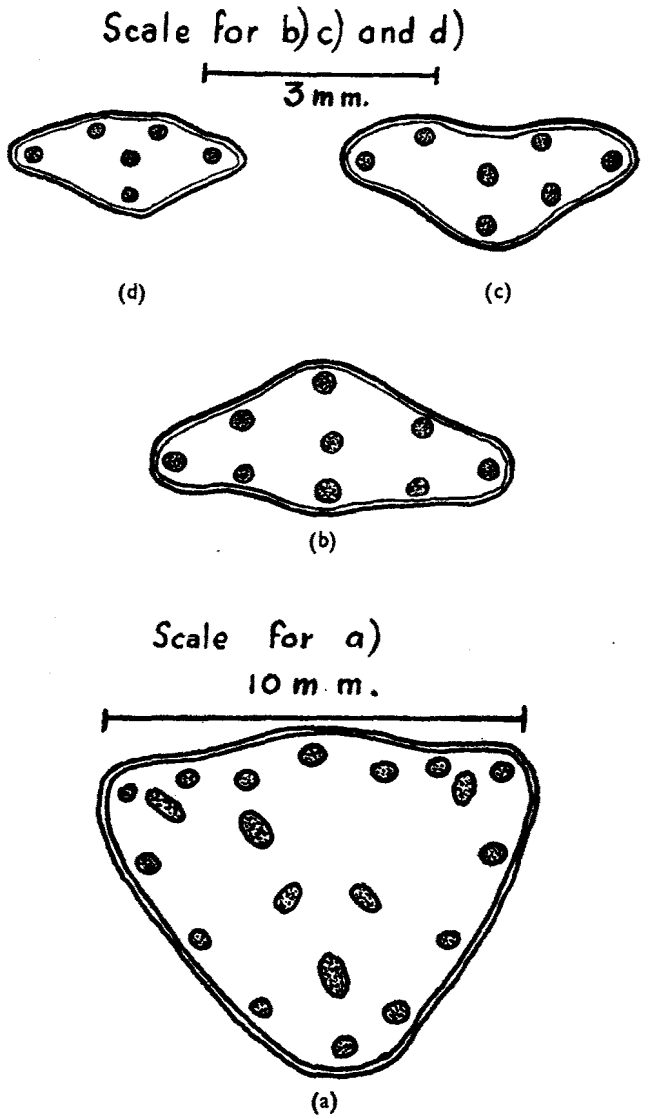

Fig. 9. Characteristic forms of petiole anatomy in British *Nuphar* taxa. (a) *N. lutea*; (b) *N. intermedia*; (c) " introgressed " *N. pumila* ; and (d) *N. pumila*. Sources of material as in Fig. 2.

Chromosome Number and Fertility of the Chartners Lough Plants

The somatic chromosome number of *N. intermedia* was determined from root-tip material of plants from Chartners Lough collected in July and fixed in Nawashin's modification of Langlet's fluid. In all of the plants examined 2n = 34. This is the first count reported for *N. intermedia*. The number 2n = 34 is the same as that determined for both *N. lutea* and *N. pumila* from continental material (Langlet and Søderberg, 1927) and is the same as that observed in British plants of *N. lutea* from Ellesmere, Esthwaite Water and Monkhill Lough, and of *N. pumila* from Shropshire, examined cytologically during the present studies. A mitotic metaphase plate from *N. intermedia* is illustrated in Fig. 10b, together with ones from *N. lutea* (Fig. 10a) and *N. pumila* (Fig. 10c), all from British sources. There appear to be no characteristics of chromosome size or morphology which would serve in any way to discriminate the three.

It has unfortunately not yet been possible to observe the course of meiosis in *N. intermedia*. That this is irregular is suggested by the low pollen fertility of the Chartners Lough plants, c. 15 per cent. This contrasts with a pollen fertility of c. 97 per cent in *N. lutea* (plants from Ellesmere), c. 85 per cent in the Avinlochan colony and c. 95 per cent in *N. pumila* (plants from Lochanovie). The average diameter of the fraction of perfect grains produced by the Chartners Lough *N. intermedia* was 45·57 $\mu \pm 0{\cdot}41$ and this is not significantly different from the pollen diameter of *N. lutea* (44·9 $\mu \pm 0.30$) although significantly greater than that of the pollen of *N. pumila* (42·87 $\mu \pm 0{\cdot}34$).

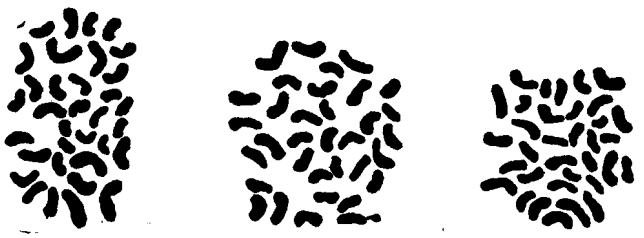

Fig. 10. Somatic chromosomes in British *Nuphar* taxa. (a) *N. lutea* from Esthwaite Water; (b) *N. intermedia* from Chartners Lough; and (c) *N. pumila* from Shropshire (× c. 3,000).

Infertility is equally well marked on the female side in *N. intermedia*. Whereas ripening of the fruit proceeds quite normally, a high percentage (c. 80 per cent) of ovules aborts, so that the loculi of the ripe fruit are very irregularly filled. Among the seeds which do set there is considerable variability in size (range in maximum length : 2·00-5·00 mm., $M = 4{\cdot}06 \pm 0{\cdot}08$). The range of variation is smaller in both *N. lutea* (range 3·50-5·00 mm., $M = 4{\cdot}96 \pm 0{\cdot}05$) and in *N. pumila* (range 2·00-4·00 mm., $M = 3{\cdot}32 \pm 0{\cdot}05$).

Attempts to germinate *N. intermedia* seed were made during 1952. Fruits from Chartners Lough were allowed to rot in water and the seeds collected immediately they were released. Germination actually began in these *N. intermedia* seeds more rapidly than those of *N. lutea* or *N. pumila* collected and ripened in the same season. Of a total of 102 seeds, 7 germinated within two months of release and initial growth was vigorous.

N. intermedia elsewhere in the British Isles : the Evidence of Herbarium Material

To supplement the study of *N. intermedia* in Chartners Lough twenty specimens of British plants referred to *N. intermedia* preserved in various herbaria have been examined. The localities from which these were collected are those indicated in Fig. 1. These herbarium specimens may be taken to represent a fairly random sample from the taxonomic unit "*N. intermedia*" as it has been interpreted by British botanists. In Table 4 the

Table 4

Meristic and size data for floral characters in British *Nuphar* taxa (*ex herbariis*; localities as in Table 3).

Taxon	Sepal		Carpel		Peduncle
	length	width	number	diameter	diameter
N. pumila	16·77 ± 0·28	10·52 ± 0·18	9·53 ± 0·29	5·62 ± 0·26	3·86 ± 0·09
N. intermedia	19·09 ± 0·22	15·11 ± 0·71	10·53 ± 0·36	6·29 ± 0·19	4·67 ± 0·27
N. lutea	29·16 ± 0·57	23·69 ± 0·70	15·58 ± 0·30	10·31 ± 0·25	6·33 ± 0·21

(All size measurements in mm.)

data obtained from them are compared with equivalent data derived from 105 specimens of British *N. lutea* and 58 specimens of British *N. pumila*. Naturally not all of the herbarium specimens have been equally favourable for the assessment of the selected attributes, but in no case do the means of Table 4 refer to less than 15 individuals for *N. intermedia*, 16 for *N. pumila* and 51 for *N. lutea*.

Direct comparison between the data for Table 4 and those of Table 1 is not permissible except in the case of carpel number, because of the considerable shrinkage resultant from drying in herbarium specimens, which ranges from 20-30 per cent. However, it is evident that the size relationships revealed by the herbarium samples of the three taxa are closely similar to those indicated by the fresh samples, the proportional differences in sepal size being almost identical (see Fig. 12). The same is true also of peduncle and carpel diameter. The correspondence of carpel number (a feature not, of course, affected by drying) between the herbarium samples and the fresh ones is quite remarkably close for *N. lutea* and *N. pumila* (Fig. 11). The difference between the means for carpel number of the herbarium and fresh samples of *N. intermedia* (1·16) is quite small, although just significant statistically (p = ·04).

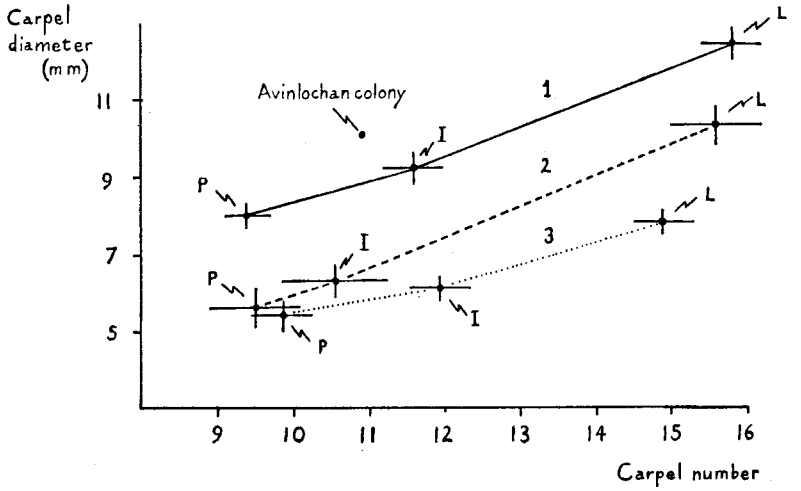

Fig. 11. The relation of carpel number to carpel diameter in European *Nuphar* taxa. Series 1 from living British plants; Series 2 from British plants *ex herbariis*; and Series 3 from continental plants *ex herbariis*. P = *N. pumila*; I = *N. intermedia*; and L = *N. lutea*. The means of each sample are inserted, and distances corresponding to twice the standard error are indicated either side of the mean. The Avinlochan colony (" introgressed " *N. pumila*) is indicated separately.

N. intermedia in Continental Europe

As with material from British sources, the specimens of the three *Nuphar* taxa from continental localities in the British Museum and Kew herbaria have been employed as samples for the purpose of a biometrical study. The numbers of plants examined were 99 for *N. lutea*, 92 for *N. pumila* and 87 for *N. intermedia*. Data from these samples comparable with those of Table 4 for British samples are given in Table 5. The same size relationship prevails between the three taxa as in the British fresh and British herbarium material (Figs. 11 and 12). The continental and British herbarium samples of *N. pumila* and *N. intermedia* are closely similar in size, carpel number, ovary diameter and peduncle diameter, none of the differences being statistically significant. In *N. lutea*, the mean values of sepal length and width for the continental herbarium sample are slightly smaller than for the British herbarium sample. The carpel numbers are

not significantly different, although the difference in the diameter of the stigmatic disc is certainly so.

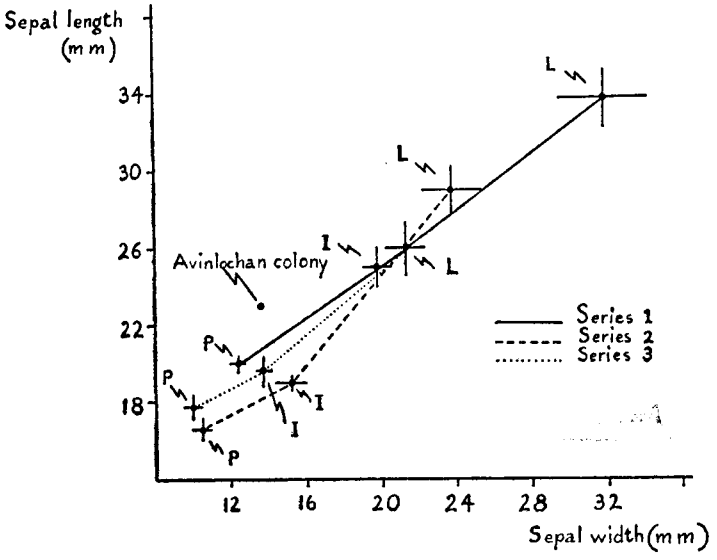

Fig. 12. The relation of sepal length to sepal width in European *Nuphar* taxa. Code as in Fig. 11.

TABLE 5

Meristic and size data for floral characters in Continental European *Nuphar* taxa (*ex herbariis*; localities as in Table 3).

Taxon	Sepal		Petal	Carpel		Peduncle
	length	width	length	number	diameter	diameter
N. pumila	17·93 ± 0·31	10·02 ± 0·21	5·85 ± 0·39	9·85 ± 0·22	5·43 ± 0·21	3·48 ± 0·08
N. intermedia	19·63 ± 0·37	13·74 ± 0·27	7·27 ± 0·22	11·96 ± 0·20	6·07 ± 0·13	3·84 ± 0·09
N. lutea	25·91 ± 0·63	21·50 ± 0·50	14·30 ± 0·39	14·93 ± 0·20	7·85 ± 0·17	6·05 ± 0·23

(All size measurements in mm.).

Robert Caspary, who conducted extensive researches on the Nymphaeaceae in the period 1855 to 1891 , accumulated a very large amount of statistical data on both *Nuphar* and *Nymphaea* in Europe, and must be considered to be the first botanist to recognise the importance of population studies in investigating the variability of these genera. The counts of petal number, stamen number and carpel number which he published (1870) for plants of *N. pumila* and *N. intermedia* have been condensed in the data given in Table 6. The " *N. pumila* aggregate " in Table 6 refers to collections of the species from six stations in the Vosges and Black Forest. The *N. intermedia* sample was also derived from localities in these areas. For *N. lutea* Caspary reports only observed ranges and these are given (together with their mid-points) in Table 6 for comparison. Caspary's *N. pumila* aggregate differs little in carpel and petal number from the British *N. pumila* aggregate, for which data are given in Table 1. While his data suggest a reasonable correspondence in carpel number between the *N. intermedia* samples he examined and that of the Chartners Lough sample, there is clearly a considerable difference in petal number, the mean in Caspary's sample being almost twice that of the Chartners sample, and actually greater than that for the aggregate British *N. lutea* sample, for which data are

given in Table 1. It is also notable that in *N. lutea* the range quoted by Caspary for continental plants (13-26) differs in the same direction from the British range of 11-20 in the aggregate sample (83 plants).

TABLE 6

Meristic data for floral parts from Continental European *Nuphar* taxa (data after Caspary 1870).

Taxon	Petal number	Stamen number	Carpel number	Total number floral parts
N. pumila	10·71 ± 0·16	46·38 ± 1·03	10·03 ± 0·05	69·08 ± 1·42
N. intermedia	18·16 ± 0·85	94·51 ± 2·20	13·18 ± 0·31	130·60 ± 2·87
N. lutea				
(*a*) midpoint	19·50	146·50	17·00	190·00
(*b*) range	13-26	86-207	10-24	130-250

In addition to his data on meristic variation in *Nuphar*, Caspary (1869, 1879) provided much information on seed and pollen fertility. For plants referred to *N. intermedia* he reported a range of pollen fertilities from 14 to 71 per cent, these being in contrast with *N. lutea* and *N. pumila*, in neither of which did he obtain plants with pollen fertility lower than 95 per cent. Seed set was correspondingly lower in the *N. intermedia* examined by Caspary, but was surprisingly variable, the mean number in colonies observed by him ranging from 5·6 to 41·7. This compares with a range of 32·7-135·1 mean numbers per fruit for *N. pumila* colonies and 93·8-418·1 for *N. lutea* colonies.

One of Caspary's (1869, 1870) most important contributions to the understanding of *N. intermedia* was to cross *N. lutea* and *N. pumila* reciprocally and bring the F_1 hybrids to flower in cultivation. Morphological comparisons of these hybrids with wild *N. intermedia* convinced him that the latter had originated in the same manner in nature. Pollen counts in the artificial hybrids showed 86·3 per cent malformed pollen (*N. lutea* female parent) and 85·6 per cent (*N. pumila* female parent). The corresponding average numbers of seeds per fruit were 14·9 and 18·4.

DISCUSSION

The status of the presumed parents

The evidence presented above suggests that while both *N. lutea* and *N. pumila* may show considerable local variability, they form reasonably homogeneous units throughout their European areas and do not intergrade to any appreciable extent. The geographical distributions of the two are at present not completely mutually exclusive, but, generally speaking, *N. pumila* has a more northerly range than *N. lutea*, and also tends to replace *N. lutea* in mountainous regions (Meusel, 1943). However, geographical and ecological isolation cannot be looked upon as the only, or even the main, factors holding the two species apart today, since, as we have seen, the experiments of Caspary, in which he crossed reciprocally typical representatives of the two, showed that, while they are interfertile enough to produce a vigorous F_1, the F_1 itself is of relatively low fertility. There is thus a partial inherent barrier to gene exchange in spite of their possession of the same chromosome number, and *N. lutea* and *N. pumila* must be looked upon as ecospecies in Turesson's terminology. It remains probable, however, that the original differentiation of the two species from their common ancestor took place under conditions of spatial isolation, and that the present distributional trends – *N. pumila* towards the north and *N. lutea* more southerly – in Europe reflect former complete vicariousness. There is no

evidence from which we can deduce the period when this geographical isolation was last complete, but there is little reason to believe that it could have been so during the post-glacial period. Differentiation of the two ecospecies may have actually been completed by late Tertiary, or it may perhaps have taken place during an interglacial. In any case, it is likely that during one or more of the Pleistocene glacial maxima the areas of both were severely compressed in southern or eastern refugia, possibly with some commingling of the populations. The subsequent immigration in the post-glacial into the formerly glaciated area would then see a " sorting out " of the ecospecies, *N. pumila* penetrating further north and becoming extinct in the south except where local microclimates favoured its persistence. The much-studied *N. pumila* populations of the Vosges and other central European mountains are no doubt relics of this nature. A similar history may be postulated for the remarkable southern colonies in the British Isles, those in Merioneth and Shropshire, if indeed the latter has not resulted from a relatively recent human introduction.

The status of N. intermedia

On the basis of the morphological evidence given above, *N. intermedia* might appear to be as homogeneous a unit in Europe as *N. lutea* or *N. pumila*. Two interpretations of this situation are possible, namely, (*a*) that it forms a separate third pure-breeding species, or, (*b*) that the plants placed under it are all hybrids of the same parentage presumably having arisen in several different localities independently. The first of these possibilities would seem to be ruled out decisively by the impaired fertility of the plants placed under *N. intermedia*. No populations are known which would fit the diagnosis of *N. intermedia* and which possess at the same time a fertility comparable with that in *N. lutea* or *N. pumila*.

This leaves the second interpretation, long accepted by systematists, that *N. intermedia* is the assemblage of hybrids from the cross *N. lutea* × *N. pumila*. Caspary's demonstration that the artificial F_1 hybrid of this parentage resembles closely plants placed under *N. intermedia* would seem to put this interpretation beyond doubt.

This ascription of a hybrid origin to *N. intermedia* does not, however, solve all the problems connected with it. The remarkable apparent homogeneity of *N. intermedia*, considered simply as a taxonomic unit, requires some explanation. The data of Tables 1, 3, 4 and 5 show that the intrinsic variability of the three aggregates of the taxon considered here, the Chartners sample, and those of British and Continental European herbarium material, is not appreciably greater than that found in comparable samples of *N. lutea* and *N. pumila*. Had the herbarium samples alone been involved, this would hardly have been a matter for surprise; " *N. intermedia* " was, after all, a taxon created to cover plants falling between *N. lutea* and *N. pumila*, and it is only to be expected that plants preserved in herbaria under this name should have been selected to be as " intermediate " as possible in character. The case of the Chartners Lough colony is somewhat different, since the sample analysed covered the full range of variation observed, there being no question of selection for plants of intermediate character.

The presumed parents of *N. intermedia* differ from each other mostly in " quantitative " characters, and this fact, and the extremely intermediate nature of Caspary's artificial hybrids, would seem to suggest a polygenic control of the characters involved. In a hybrid colony containing generations later than the F_1, one might expect some degree of segregation, and certainly a wider range of variation than in " pure " colonies of either parent. The relatively narrow variation range of the Chartners colony, and others like it, indicates either that subsequent generations to the F_1 are *not* present, or that some form of selection is acting to ensure the survival only of plants near the F_1 mode (Fig. 5).

The possibility that colonies like that in Chartners Lough are in fact ancient clones derived from a single original F_1 plant cannot be dismissed from consideration. The

capacity of *Nuphar* to reproduce vegetatively is well known. The bottom of Chartners Lough in the area of the *Nuphar* is entirely covered with entangled rhizomes, and although verification would appear now to be impossible, it does not seem inconceivable that all are ramifications of a single huge plant. If so, the age of this plant must be enormous, far exceeding the century or so for which individual *Nuphar* plants have been observed to persist in cultivation.

No seedlings were noted in the Chartners Lough colony, and it is quite obvious that, under the existing conditions, seedlings would find considerable difficulty in establishing themselves against the competition of the mature plants, particularly for light. Nevertheless the successful germination of seeds from the fruits of the season of 1952 shows that propagation by this means is at least feasible. The low pollen and ovule fertility points to the probability that severe meiotic irregularities are present, arising possibly from structural differences between the parental chromosomes. If so, it is possible that genetical recombination is severely limited, and that only those spores survive and function which approach a particularly favourable genetical balance.

It may be that the uniformity of such colonies as the Chartners one is maintained by a combination of several factors – by the extensive occurrence of vegetative propagation of successful plants, by the fact that only a limited amount of genetical recombination is possible, and by the stringent elimination, through competition, of ill-adapted seedlings.

Anomalous populations

A comment on the Avinlochan colony seems appropriate at this point. This colony has usually been accepted by systematists as falling within the range of *N. pumila*, and was so in the course of the present study until it was realised that it combined a relatively low pollen fertility with certain anomalous features not encountered in other populations of *N. pumila*. Accordingly, the biometrical data relating to this colony have been kept separate in the tables and not incorporated in the *N. pumila* aggregate. Considering all of these data, it will be seen that the Avinlochan colony does, in fact, in its morphological features depart somewhat from the other colonies of *N. pumila* in the direction of *N. lutea*. This and the depressed fertility would seem to indicate that it has in the past suffered genetical contamination from *N. lutea*. The ovary fasciation illustrated in Fig. 6 is present to a greater or lesser degree in the bulk of individuals of the colony, and must be looked upon as sub-pathological. A similar, but even more extreme, example of the incidence of ovary fasciation of this type was encountered by Caspary (1870) in a colony, referred to *N. pumila*, in Titisee. Caspary's data suggest that there is a considerable amount of variation within and amongst the *Nuphar* populations of the lakes of the Vosges and the Black Forest, and here again the possibility of different degrees of introgression of *N. pumila* and *N. lutea* is present. The great variation in pollen fertility observed by Caspary in these colonies points in the same direction.

The distributional problem of N. intermedia

The final problem concerns the existence of *N. intermedia* in localities like Chartners in isolation from one or both parents – a circumstance, in the case of the Chartners colony, once considered to rule out the possibility of its being of hybrid origin (Baker and Tate, 1868). Once again there is more than one possible explanation. The simplest would be that colonisation has arisen relatively recently from a chance hybrid seed carried by some agency – presumably water-fowl – from a locality where the two parents exist today in close proximity. While this explanation cannot altogether be rejected, it would seem improbable. With the Chartners colony the minimum distance of transport would have to be of the order of 80-90 miles and it would seem to be pressing the idea of chance

long-range dispersal rather far to assume that all of the hybrid colonies marked in Fig. 1 have arisen in this manner.

The alternative explanation, that the isolated southern *N. intermedia* colonies originated in their present stations at the time when the two parental species occurred together in the neighbourhood, would seem to be more probable. It is likely that *N. pumila*, like other boreal and montane species, has been in the process of area-contraction for some considerable time. It can hardly be doubted that *N. pumila* did exist in N. England at one time during the early post-glacial period, but without fossil evidence it is difficult to say how long it is likely to have persisted in the Cheviot area. Two factors probably led to its extinction: the climatic amelioration and the gradual elimination of suitable habitats through the growth of peat bogs. Smythe (1930) has suggested that Chartners and the other remaining lakelets on the Northumberland Fells may have arisen as moraine-dammed lakes in the closing stages of the last glaciation; how many others in the area once open to colonisation by *N. pumila* have now entirely disappeared we have no means of knowing.

N. lutea, although no doubt a later immigrant into the British Isles than *N. pumila*, is known to have been present in early boreal times; reproductive contacts with *N. pumila* may have taken place repeatedly during the replacement of one by the other in southern and lowland districts. Where in the neighbourhood of expanding populations of *N. lutea*, the hybrids probably became eliminated by competition and genetical " swamping." In a few more isolated localities, particularly in relatively upland stations where their " intermediate " genotype may have been of adaptative value, they appear to have been able to persist.

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