TAXONOMICAL AND ECOLOGICAL STUDIES IN CALTHA PALUSTRIS L.

(PRELIMINARY REPORT)

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I. INTRODUCTION

The genus *Caltha* comprises approximately 12–16 species growing in wet, marshy places in arctic and North and South temperate regions. The genus in classed in the tribe *Helleboreae* of the *Ranunculaceae*. A. DE CANDOLLE (1818) subdivided the genus into two sections: *Psychrophila* and *Populago*, the distinguishing features being that in the former the calyx is persistent, the auricles of the leaf laminae having upturned to erect appendages, whereas in the latter the calyx is deciduous and the leaves are cordate to reniform. In the section *Psychrophila* he placed two species of the Southern Hemisphere, the other section including all the species of the Northern Hemisphere. The peculiar leaf characters of the species of the Southern Hemisphere (5–8) are highly distinctive.

Not only the morphological characters of some species of the genus vary considerably, but also in terms of cytology differentiation within the genus and even within species occurs. Therefore, various authors differ in that opinion with regard to the taxonomic treatment of the many forms.

In 1854 SCHOTT, NYMAN and KOTSCHY subdivided *Caltha palustris* into the species *C. cornuta*, *C. latifolia*, *C. laeta*, *C. intermedia*, *C. vulgaris* and *C. alpestris*. BECK (1886) also split *Caltha palustris* into several species, but his delimitation differs from that of Schott, Nyman and Kotschy. HUTH (1892) in his "Monographie der Gattung *Caltha*" did not agree with these authors for the following reasons: 1. the common areas of all these forms; 2. the numerous transitional forms between the different types; and 3. the large variability of individuals of the same population. REESE (1954), and TUTIN in Heywood, Flora Europaea I (1964), are also of the opinion that it is very difficult to classify the different forms of *C. palustris* satisfactorily.

In *Caltha palustris* sensu lato various chromosome numbers have been reported. Two euploid series can be distinguished: 2n=16–32–48–64,

$2n = 28$–56. Also aneuploidy occurs: $2n = 32 \pm 3$; 49; 50; 53–60. (Cf. Löve and Löve, 1961). Reese (1954) reported the occurrence of B-chromosomes in *C. palustris*. In view of the unsatisfactory classification of many forms of *Caltha palustris* L. s.l. it seemed worthwhile to undertake combined cytological and morphological studies in this species-complex. In the first place attention was given to the Dutch material, but the author intends to extend her studies to material originating from other parts of the world. Ecological problems will be also taken into consideration, as well as crossing experiments. Other species of the genus *Caltha* will also be the object of further investigations.

II. Material and methods

The plants studied were collected in their natural habitat, the greater part from the Netherlands, but also some populations from other European countries. From each population 5–20 plants were dug out and transferred to the experimental garden; of the same population 5 plants were dried. In most populations a description of the vegetation was made according to the method of Braun-Blanquet. Voucher specimens of all plants have been deposited in the herbarium of the State University of Utrecht. The collected plants are growing under the same conditions in the experimental garden and will be used for comparative morphological studies and for crossing experiments.

The determination of the chromosome numbers was based on the study of roottip mitoses.

In the preliminary stage microtome sections of 15 μ were used, stained according to Heidenhain's haematoxylin method. The results were very poor. Therefore squash slides were made. As the chromosomes in *Caltha palustris* are very long it was necessary to find a method to decrease their length. Neither cold treatment nor oxychinolin yielded satisfactory results. The colchicin method, modified after Reese (1954), proved to be more satisfactory. There was a pre-treatment for 4 hours of roottips of 2 cm length in a 0,1 colchicin solution. In this solution the chromosomes proved to be spread sufficiently, and their length decreased to ± 50 % of the original size. After this treatment the roottips were fixed in Carnoy, hydrolysed in $_1$N HCl at 58–60 °C for 20 minutes, and stained with leucofuchsin (Feulgen) for one hour. Plastic coverslips were used and then removed in aceton one hour after squashing.

III. Results

III.1. *Chromosome numbers*

The chromosome numbers of the populations studied are listed in table 1., together with the collection number, the number of the vegetation description (record), and the place of origin of the material. In one plant of every population the chromosomes of 5–10 metaphase plates were counted; in the others the counting of two metaphase plates seemed to be sufficient.

TABLE 1

List of the specimens examined cytologically, with the number of chromosomes, reference to the collection number and number of the record and the origin of the material.

Coll. no.	Record no.	2n	Origin of the material
D108–130	65–7	32	Denekamp, wet pasture (Overijssel).
D131–140	65–8	32	Denekamp, wet forest (Overijssel).
D151–160	65–9	32	Springwood near Gulpen (Limburg).
D161–166	65–10	32	Pasture near Gulpen (Limburg).
D167–174	65–11	32	Former riverbed of the Geul near Epen (Limburg).
D175–182	65–12	32	Springwood near Epen (Limburg).
D195–206	65–16	32	Woudhuis near Apeldoorn (Gelderl.).
D207–226	–	32	Kotten near Winterswijk (Gelderl.).
D254–285	65–19	32	Bank of the river IJssel, near Ellecom (Gelderl.).
2104–2120	65–19	32	Idem.
2626	–	32	Terziet (Limburg).
D321–324	66–3	32	Marsh in the valley of the river Geul, South of Epen (Limburg).
D403–407	–	32	Korenburgerveen near Winterswijk (Gelderland).
D141–150	–	32	Germany: Bentheim.
D325–333	–	32	Spain: between la Corũna and El Ferrol.
D341–343	–	32	Portugal: Pitões.
D409	–	32	France: Cévennes.
D410–419	–	32	France: Lac Mt. Cenis, peatbog (Haute Savoie).
D420–424	–	32	Idem.
D439–445	–	32	France: Lac Mt. Cenis, bank of a rivulet.
B163	–	32	Finland, Rovaniemie.
D1–5	–	56	Tienhoven (Utrecht).
D6–10	65–1	56	Johannapolder near Utrecht (Utrecht).
D10–40	63–054	56	Oost-Voorne, former airstrip (Zuid-Holland).
D41–54	–	56	Oost-Voorne, Gamandervallei (Zuid-Holland).
D55–63	65–3, 65–4	56	Oost-Voorne, Quackjeswater (Zuid-Holland).
D66–78	65–5	56	Hoogeweg, N.W. Overijssel (Overijssel).
D79–94	65–6, 65–6A	56	Coast of the IJsselmeer near Nunspeet (Gelderl.).
D95–102	–	56	By the river Lek near Utrecht (Utrecht).
D103–107	65–1	56	Johannapolder near Utrecht (Utrecht).
D183–194	65–13	56	Molenpolder near Utrecht (Utrecht).
D227–235	–	56	Pasture near Waddendike, Isle of Terschelling.
D240–249	65–17	56	Wet dune valley, east part of the Isle of Terschelling.
D250–253	–	56	Vuntus near Utrecht (Utrecht).
2379–2383	65–18	56	Brabantse Biesbosch near Raamsdonk (N.-Brabant).
1800–1845	–	56	Coast of the IJsselmeer between Baarn and Spakenburg (Utrecht).
1852–1860	66–14	56	Marsh near the Grebbeberg, near Rhenen (Utrecht).
D301–310	66–1	56	Ulvenhoutse Bos near Breda (N.-Brabant).
D311–317	66–2	56	Annabos near Breda (N.-Brabant).
D318–320	–	56	Oost-Voorne, Quackjeswater (Zuid-Holland).
D348–357	66–5	56	Coast of the IJsselmeer near Ermelo (Gelderl.).
D358–363	66–6	56	Coast of the IJsselmeer near Elburg (Gelderl.).
D364–369	66–7	56	Idem.
D370–376	66–9	56	Helsbroek near Tilburg (N.-Brabant).
D377–381	66–10	56	Idem.

TABLE 1 *(continued)*

Coll. no.	Record no.	2n	Origin of the material
D382–385	–	56	Along the Wilhelminakanaal near Tilburg (N.-Brabant).
D386–397	–	56	Sliedrechtse Biesbosch near Dordrecht (Zd.-Holland).
D398–402	–	56	Coast of the IJsselmeer near Putten (Gelderl.).
D446–447	–	56	Tienensloot near Sneek (Friesland).
D458–463	66–13	56	Bennekomse Meent near Wageningen (Gelderl.).
D334–340	–	56	Spain: Mirador de la Reina, alt. 1100 m.
D343–347	–	56	Spain: Sierra de Covadongo, alt. 600 m.
D425–428	–	56	France: Col de l'Iseran, alt. 2100 m. (Haute Savoie).
D429–438	–	56	France: waterfall near Bonneval sur Arc (Haute Savoie).
D448–457	–	56	Austria: bank of the Wolfgangsee.
D300A–300E	–	44	Cultivar: C. palustris ? var. alba.

TABLE 2

A survey of the cytological investigations in the species *Caltha palustris* s.l.

Species	2n	Author	Origin of the material
C. palustris L.	32	Langlet (1927)	Sweden
,,	32, 48, 56	Sokolovskaya and Strelkova (1938, 1941)	N. Europe
,,	32	Gregory (1941)	N. America
,,	32	Felföldy (1947)	Hungary
,,	28	Löve and Löve (1948)	N. Europe
,,	32	Polya (1950)	Hungary
,,	32	Leoncini (1951)	Italy
,,	32+0–3, 53–60	Reese (1954)	Germany
,,	32	Dahl and Rowley (1956)	Minnesota, U.S.A.
,,	32, 56	Sokolovskaya (1958, 1962)	Sovjet Union
,,	32	Satczek, in Skalinska et al. (1959)	Poland
,,	28	Sorsa (1962)	Finland
,,	56	Kootin Sanwu (1964)	Great Britain
C. arctica R.Br.	56	Bormann and Beatty (1955)	Alaska
,,	56	Sokolovskaya (1958)	Sovjet Union
C. cornuta S.N. et K.	16	Leoncini (1951)	Italy, cult.!
,,	24, 30–52	Maugini (1957)	Italy
,,	56	Satczek, in Skalinska et al. (1959)	Poland
C. laeta S.N. et K.	32	Langlet (1927)	Sweden
,,	32	Delay (1947)	Unknown
,,	32, 54–64	Leoncini (1951, 1952)	Italy
,,	32	Maugini (1953)	Italy
,,	56	Satczek, in Skalinska et al. (1959)	Poland
C. radicans Forst.	48–0±2B	Löve and Löve (1948)	Scotland
,,	56	Kootin Sanwu (1964)	Great Britain
C. fistulosa Schipcz.	32	Kurita (1956)	Japan

Up till now in the field no intermediates between 2n = 32 and 56 have been found. Hybridization experiments are planned in order to investigate the crossability of the cytotypes. The results reported in this chapter will be discussed further in the chapters III.2 and III.3, in connection with morphological and ecological data.

Table 2 gives a survey of the chromosome numbers of *Caltha palustris* s.l. as reported in the literature.

III.2. *The relation between morphological and cytological characters*

In the Netherlands two cytotypes of *Caltha palustris* were found, 2n = 32 and 2n = 56. The relation between the number of chromosomes and some morphological characters, such as habit, size of the pollengrains, number of carpels, number of flowers of the inflorescence, height of the plant, leaf shape and margin, number of stamens, and number of petals, were studied more in detail.

The habit

Both in the field and in the experimental garden one of the first things which catches the eye is the fact that there are erect and prostrate plants. In the same population both types of plants may be found, but generally the whole population tend to be either erect or prostrate. Of ten populations of 2n = 32 five populations had mainly prostrate plants, two were erect to prostrate, and three had only erect plants. Of 15 populations of 2n = 56 ten populations had erect plants, four erect and prostrate plants, and one population had only prostrate plants. Summing up, there seems to be a tendency for a prostrate habit in the Dutch populations of the cytotype 2n = 32 and for an erect habit in the cytotype 2n = 56, but there is a certain degree of overlap. Figs. 1, 2 and 3 demonstrate these phenomena.

The pollengrains

The mature pollengrains of flowers fixed in aethanol 70 % were dispersed in saffranin. 1200 Pollengrains from 12 flowers were measured, 100 from each, representing 5 populations each of both cytotypes. From the data of every 100 pollengrains the mean ($\bar{x}$) and the standard deviation (S.D.) were calculated. For comparison of the size of the pollengrains of both cytotypes also the standard error of difference (S.E. of D.) was determined. Table 3 gives a survey of the size of the pollengrains of both cytotypes.

The standard error of difference is 0,03, the observed difference between the diameter of 2n = 32 and 2n = 56 is 4,88 μ. This is much more than twice the standard error of difference, being 0,06 μ, therefore we conclude that although the ranges of variation overlap in the two cytotypes it is highly probable that the cytotypes with 2n = 56 chromosomes have larger pollengrains. Fig. 4 gives an impression of the extent to which the ranges of variation of the two cytotypes overlap. (See page 7).

TABLE 3

Pollengrain diameter of 1200 pollengrains of *Caltha palustris*, of the cytotypes 2n=32 and 2n=56. N. for every number is 100

Population no.	2n	diameter (μ) $\bar{x} \pm$ S.D.
D108–120	32	23,44 ± 0.47
D108–120	32	23,97 ± 0,61
D254–285	23	22,27 ± 0.52
D410–419	32	22,92 ± 0,44
D195–206	32	22,82 ± 0,51
D167–174	32	23,52 ± 0,64
Average	32	22,99 ± 0,53
D227–235	56	28,15 ± 0,47
D227–235	56	31,86 ± 0,69
D240–249	56	26,87 ± 0,37
D78–88	56	27,50 ± 0,31
D183–194	56	26,83 ± 0,42
2379–2383	56	26,01 ± 0,46
Average	56	27,87 ± 0,45

Number of carpels

In 40 plants of both cytotypes the number of carpels of one flower was counted. The true mean of the sample 2n=32 was 9 carpels and of the sample 2n=56 12 carpels (see also table 4).

Diameter of the flowers

Also in this case 80 flowers of 80 different plants were measured, equally distributed among the two cytotypes (see table 4).

Number of flowers per inflorescence

In 40 inflorescences of 40 plants with 2n=32 the number of flowers was counted (also 40 plants 2n=56, see table 4).

Height of the plants

In 40 plants each of the two cytotypes the height of the fruitbearing plants was measured. (See table 4).

TABLE 4

Survey of the number of carpels in each flower, the flower diameter, the number of flowers per inflorescence, and the height of the fruit-bearing plants

	$\bar{x}_{32} \pm$ S.D.	$\bar{x}_{56} \pm$ S.D.	$\bar{x}_{56} - \bar{x}_{32}$	2×S.E. of D.
No. of carpels	8,99 ± 2,63	12,24 ± 4,70	3,25	1,16
Diam. of flowers (mm.) .	36,13 ± 7,02	40,61 ± 6,17	4,48	2,06
No. of flowers per inflorescence	3,93 ± 2,46	6,85 ± 2,63	2,92	1,22
Height of the plants. . .	28,83 ± 13,88	38,12 ± 17,91	9,29	7,62

Fig. 1: D135 2n=32 and D6 2n=56.

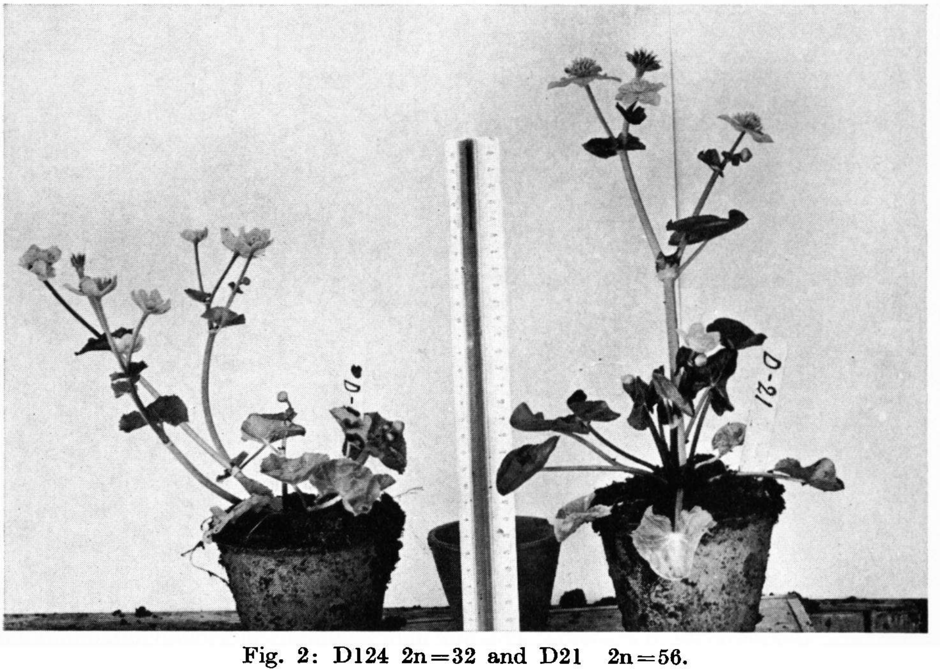

Fig. 2: D124 2n=32 and D21 2n=56.

Figs. 1, 2 and 3: Habit of different plants.

The 2n=32 plants are prostrate, the 2n=56 erect, these figures also show the larger size of the 2n=56 plants.

Fig. 3: D218 2n = 32 and D2381 2n = 56.

According to table 3 and 4 a correlation between the chromosome number and the morphological characters seems to exist. The conclusion may be drawn that in the Dutch populations studied the number of carpels, the diameter of flowers, the number of flowers, and the height of the plants are smaller in the 2n = 32 than in the 2n = 56 plants.

A correlation between the degree of polyploidy and the number of stamens per flower does not exist; the number fluctuates in both cytotypes between 85 and 167. The size of the leaves and the shape of the leafmargins also show considerable variation. In both cytotypes the measurements of

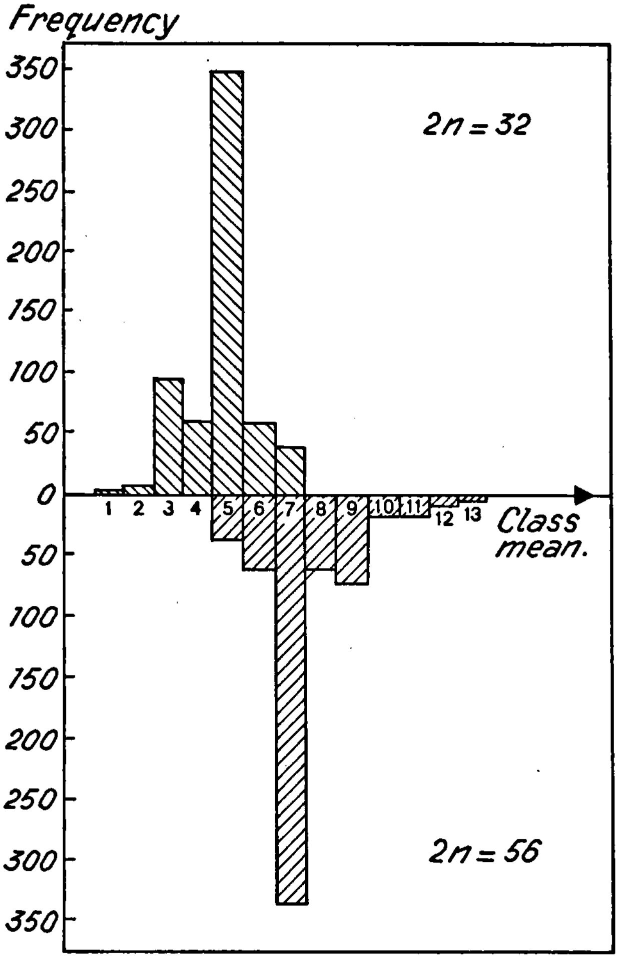

Fig. 4. Diagram of the diameter of the pollengrains of two cytotypes of *Caltha palustris*, at the top 2n=32, below 2n=56.

Class mean in μ:

1=15,6 μ	5=23,4 μ	9=31,2 μ	13=39 μ
2=17,6 μ	6=25,4 μ	10=33,2 μ	
3=19,5 μ	7=27,3 μ	11=35,1 μ	
4=21,5 μ	8=29,3 μ	12=37,1 μ	

length and width vary from 1,5 × 2,5 cm (small leaves) to 6 × 11 cm (largest leaves). The number of petals also varies: 70–95 % of the flowers of plants with 2n = 32 have 5 petals, the remaining 5–30 % have 6 or 7 petals. In the cytotype 2n = 56 30–5 % of the flowers have 6,7 or 8 petals.

III.3. *The relation between cytological characters, geographical distribution, and ecological preference*

In the Netherlands *Caltha palustris* grows in moist and meso-oligotrophic places, provided that the NaCl content is not too high. Caltha is characteristic for humid pastures and meadows, borders of ditches, peat-bogs, and springwoods. The plant never grows in places which are completely dry in the summer. In the fresh water delta the Biesbosch ZONNEVELD (1960) regards *Caltha* as a characteristic plant of *Phragmites*-fields, with its actual optimum in a strongly reduced substrate with poor soil aeration. *Caltha* disappears under strong competition by this reed. But on a strongly reduced, soft soil the vitality of *Phragmites* is greatly diminished and then dense stands of *Caltha* can develop.

Up till now plants with the chromosome number 2n = 56 have been met with in the western and central provinces of the Netherlands (holocene), whereas in the eastern provinces, as well as in Limburg (pleistocene) plants with 2n = 32 have been found. (See also Fig. 5).

Table 5 gives the phytosociological analysis, with on the right side the records of plants with the chromosome number 2n = 32 and on the left those with 2n = 56.

The principles of the Braun-Blanquet system of classification in general have been used, but we have worked with species groups consisting of species which in the literature were partly known as characteristic, companion, or casual species. In this system *C. palustris* seemed to be a species with its optimum in the alliance *Filipendulo-Petasition* because of its larger abundance/dominance in records with many species of this alliance. The cytotype 2n = 56 occurs in records on acid, not too oligotrophic or eutrophic soils, together with many species of the order *Phragmitetalia* (reedmarsh vegetations). Intermediate are the records in pastures which have many species of the class *Molinieto-Arrhenatheretea*. The cytotype 2n = 32 occurs on neutral to alcaline soils, together with many species of the *Fagetalia* and also more species of the *Filipendulo-Petasition*, an alliance of tall herbs. The records with plants 2n = 32 are often from springwoods with relatively more trees and shrubs than the records with 2n = 56 plants. In general striking differences between the cytotypes with regard to the surrounding vegetation could be demonstrated; it seems that vegetations on disturbed boggy substrates have the 2n = 56 cytotype of *C. palustris*, the river and brookmarshes and springwoods the 2n = 32 cytotype. Further investigations in this field are necessary.

TABLE 5: Records of Dutch *Caltha* populations, on the right 2n=32, on the left 2n=56.

No. of record	65–3	65–4	65–18	65–6A	65–13	53–76	66–11	66–5	65–1	63–054	65–17	66–9	66–10	66–14	66–13	66–7	66–2	65–5	66–6	65–6	65–7	65–19	65–8	66–4	65–10	65–11	65–12	65–16	65–9
Date 1965, *1966*	14–IV	14–IV	?–V	20–IV	15–VI	?	*21–VII*	*1–VII*	12–IV	?	26–V	*14–VII*	*14–VII*	*21–IX*	*15–IX*	*1–VII*	*3–V*	18–IV	*1–VII*	20–IV	30–IV	19–VI	30–IV	*5–VI*	5–V	5–V	5–V	20–V	5–V
Surface (sq.m.)	20	25	18	16	100	8	5	5	40	25	40	12	16	9	25	3	9	10	16	4	100	20	30	16	16	25	12	8	20
Watersurface (%)	30	90	0	90	0	?	30	0	30	50	70	60	0	25	60	4	0	0	0	0	0	10	20	40	60	70	<10	mud	16
Layer of herbs, covering (%)	60	30	60	15	90	100	80	100	70	100	60	100	100	90	90	100	100	80	100	90	90	100	70	100	80	40	65	20	50
Layer of herbs, height (cm)	50	40	?	25	60	?	75	100	40	90	80	80	60	50	50	100	75	15	20	15	30	40	30	100	50	50	40	60	50
Layer of trees, covering (%)	—	—	—	—	60	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15	—	90	20	—	20	50	75	—
Layer of trees, height (m)	—	—	—	—	8	?	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	20	7	—	8	12	8	—
No. of the spec. not listed	3	2	3	1	4	7	7	5	1	7	1	1	2	2	2	3	2	0	0	4	6	5	3	2	2	3	3	0	4
CALTHA PALUSTRIS	+.2	1.2	2.2	3.2	+.2	1.2	1.2	2–3.2	2.2	1.2	1.2	1.2	2.2	1.2	2.2	1.2	1.2–3	1.2	2.2	1.2	3.2	1.2	3.2	3.2	4.2	2.2	1.2	2.2	1.2
Species of the																			2n=56 ←		→	2n=32							
Ord. *Phragmitetalia*																													
Glyceria maxima	—	—	—	—	—	—	—	4.3	3.2	—	3.1	2.2	+.2	2.2	2.1–2	—	—	1.1	1.2	—	—	—	—	—	—	—	—	—	—
Equisetum fluviatile	—	—	—	—	1.1	—	2.1	—	3.1	—	—	—	—	—	—	+.1	1.1	—	—	—	—	—	—	—	—	—	—	—	—
Phragmites communis	+.1	—	—	1.2	1.2	—	—	—	—	3.5	3.1	2.2	—	1.2	—	2.1	1.1	+.1	—	+.1	—	—	—	1.1	—	—	—	1.1	—
Iris pseudacorus	1.2	1.2	—	+.1	3.2	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	+.1	—	—	—	1.2	—
Rumex hydrolapathum	+.1	—	—	—	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Scirpus lacustris	2.1	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sium erectum	—	—	1.2	3.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Myosotis scorpioides	—	.1–2	—	—	—	2.3	1.2	1.2	1.1	—	—	—	—	+.1	1.2	1.2	—	—	—	—	+.1	+.1	—	—	+.1	—	—	—	+.1
Galium palustre	—	1.1	3.5	2.1	—	—	2.2	1.1	—	—	2.1	—	—	1.1	1.1	—	—	—	—	—	—	—	—	—	1.1	—	—	—	—
Mentha aquatica	—	1.1	—	1.1	—	—	1.2	—	1.1	2.1–2	1.1	3.3	2–2	+.1	—	—	—	—	—	—	+.1	1.1	+.1	—	1.1	—	—	—	+.1
Ord. *Magnocaricetalia*																													
Ranunculus lingua	—	+.1	—	—	—	—	—	—	—	—	—	+.1	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carex paniculata	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.2
Carex acuta	—	—	—	—	—	—	—	—	—	—	—	2.2	—	1.2	4.1–3	+.2	—	—	—	—	—	—	—	3.2	—	—	—	—	3.1
Carex riparia	2.2	—	—	—	2.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Species of the Cl.																													
Molinieto-Arrhenatheretea																													
Holcus lanatus	—	—	—	—	—	—	1.2	—	+.1	—	—	—	—	—	—	—	3.2	3–4.2	2.2	—	2–32	—	—	—	1.2	—	—	—	—
Rhytidiadelphus squarrosus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	—	—	—	—	—	—	—	—	—	—	—
Plantago lanceolata	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	+.1	—	+.1	—	—	—	—	—	—	—
Cardamine pratensis ssp. prat.	—	—	—	+.1	—	+.1	—	—	1.1	—	1.1	+.1	+.1	—	1.2	+.1	1.1	1.1	—	1.1	+.1	+.1	—	—	2.2	—	1.1	—	—
Cardamine pratensis ssp. pal.	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	—	+.1	+.1	—	+.1	—	—	—	—	—	—	—	—
Rumex acetosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	1.1	1.1	2.1	—	+.1	1.1	1.1	—	—	—	—	—	—	—
Ranunculus acer	—	—	—	—	—	—	1–2.2	+.1	+.1	—	+.1	+.1	—	—	2.2	1.1	1.1	1.1	+.1	—	2.2	2.1	—	—	+.1	—	—	—	—
Anthoxanthum odoratum	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—	—	1.2	—	1.1	—	2.2	—	1.1	—	—	—	—	—	—	—
Cerastium holosteoides	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	+.1	+.1	1.1	—	1.1	—	—	—	—	—	—	—
Festuca pratensis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.2	—	—	2.1	—	—	—	—	—	—	—
Bellis perennis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	+.1	1.1	—	—	—	—	—	—	—	—	—
Trifolium repens	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	2.1	1.1	2.1	—	—	—	—	—	—	—
Trifolium pratense	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	1.1	—	—	—	—	—	—	—	—	—
All. *Molinion coeruleae*																													
Thalictrum flavum	—	—	—	+.1	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Achillea ptarmica	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	+.1	—	—	—	—	—	+.1	—	—	1.1	—	—	—	—	—
Juncus effusus	—	—	—	—	—	—	—	—	—	—	—	—	—	3–4.2	+.2	—	—	—	—	—	+.1	1.1	1.2	—	1.1	—	—	—	—
Cirsium palustre	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—	—
Lychnis flos-cuculi	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	+.1	—	—	+.1	1.1	—	1.1	—	—	1.1	—	—	—	—
Lysimachia vulgaris	—	—	—	—	—	—	—	—	—	—	—	—	1.1	+.1	—	—	—	—	—	—	—	—	—	2.2	—	—	—	1.1	—
Lotus uliginosus	—	—	—	—	—	—	—	—	—	—	—	+.1	—	4.3	—	—	—	—	—	—	—	1.1	—	1.2	—	—	—	—	—
All. *Cirsieto-Molinietum*																													
Cirsium dissectum	—	—	—	—	—	—	—	—	—	—	—	—	1.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carex hostiana	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—
Carex panicea	—	—	—	—	—	—	—	—	—	—	—	—	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agrostis canina	—	—	—	—	—	—	—	—	—	—	—	2.2–3	2.2	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—
Species of the Cl.																													
Caricetalia fuscae																													
Eleocharis palustris	—	—	—	—	—	—	—	—	1.1	1.1–2	1.1	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hydrocotyle vulgaris	—	—	—	—	—	—	—	—	—	1.1	2.1	—	1.1	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	—
Ranunculus flammula	—	—	—	—	—	—	—	—	—	1.1	—	+.1	+.1	—	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Salix repens	—	—	—	—	—	—	—	—	—	+.1	1–2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
All. *Filipendulo-Petasition*																													
Filipendula ulmaria	—	—	—	—	2.1	—	—	+.1	—	—	—	—	1.1	2.2	1.2	—	1.1	—	—	—	1.1	—	3.2	3.2	2.1	1.1	2.2	1.1	1.2
Angelica sylvestris	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	+.1	1.1	—
Valeriana officinalis	—	—	—	—	—	+.1	—	—	—	—	—	—	—	1.1	—	—	3.1	—	—	—	—	—	1.1	—	1.1	+.1	—	1.1	—
Eupatorium cannabinum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.2
Scirpus sylvaticus	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	—	—	—	—	—	+.1	2.1	—	—	—	—
Lythrum salicaria	—	—	1.2	—	—	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—
Species of the Ord.																													
Populetalia, All. *Alno-Ulmion*																													
Poa trivialis	—	—	—	—	—	1.2	2.2	—	+.2	—	—	1.2	—	—	2.2	—	—	—	—	—	2.2	2.2	—	3.2	3.2	+.2	1.2	—	—
Symphytum officinale	—	—	—	—	1.1	+.1	—	1.1	—	—	—	—	+.1	+.1	—	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—
Phalaris arundinacea	—	—	—	—	1.2	—	—	+.2	—	—	—	—	—	—	—	2.1	—	—	2.2	—	—	—	—	3.2	—	—	—	—	—
Ranunculus repens	—	—	—	—	—	3.4	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	1.1	—	—	—	—	—
Glechoma hederacea	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	—	1.1	+.1	—	+.1	—	—	—	—	—	—	1.1	—	—	—
Lysimachia nummularia	—	—	—	—	—	—	—	2.2	1.1	—	—	+.1	—	—	3.2	—	—	+.2	—	—	1.1	+.1	—	—	1.1	—	1.1	1.1	—
Urtica dioica	—	—	—	—	4.5	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	—	+.1	—	+.1	2.2	1.1	1.1	+.1
Salix spec.	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	2–3.1	—	—	2	—	—	—
Ranunculus ficaria	—	—	—	—	—	—	—	—	2.1–2	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	—	+.1	—	1.2	—	—
Species of the Ord.																													
Fagetalia, All. *Alno-Padion*																													
Alnus glutinosa	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	+.2	—	—	—	1.1	—	+.1	2	+	2	3	2	1
Equisetum telmateia	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	1.1
Cardamine amara	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	1.2	—	—
Equisetum palustre	—	—	—	—	—	—	—	+.1	—	—	—	1.1	1.1	1.1	1.1	—	—	—	1.1	—	+.1	2.1	—	2.1	2.1	—	—	—	—
Crepis paludosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	+.2	—	—
Glyceria fluitans	—	—	—	—	—	—	3.2	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	2.1	1.1	—	1.2	—	—	1.2	—
Circaea lutetiana	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—
All. *Carpinion*																													
Adoxa moschatellina	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	+.1	—
Primula elatior	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	+.1	—	2.2	—	—
Melandrium rubrum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	1.1	—	—	—
Lamium galeobdolon	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—
Polygonatum multiflorum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—
Melica uniflora	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—
Acc.																													
Calamagrostis canescens	—	—	—	—	4.5	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Poa pratensis	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—	—	3.2	2.1	—	3.2	3.2	—	—	—	—	—	—	—	—	—
Rumex obtusifolius	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	1.1	—	—	+.1	—	—	—	—
Epilobium parviflorum	—	—	—	—	—	—	—	+.1	—	+.1	+.1	—	—	—	—	—	—	1.2	—	—	—	—	—	+.1	—	—	—	—	—
Taraxacum officinale	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	+.1	—	+.1	1.2	1.1	+.1	—	—	—	—	—	—	—	—

IV. Discussion

Up till now 46 populations in the Netherlands have been investigated. With regard to the cytological studies the question arises wether certain "microspecies", distinguished, i.a. by Schott, Nyman and Kotschy are characterized by a distinct chromosome number. In some papers chromosome numbers have been reported for some microspecies (See also table 2). However, the investigations in the Netherlands indicated that despite the differences in the chromosome number the morphological differences are not sufficient to justify the separation of two species. At least in the Netherlands it seems that the classifications of Schott, Nyman and Kotschy and of Beck cannot be used. In the same population the vari-

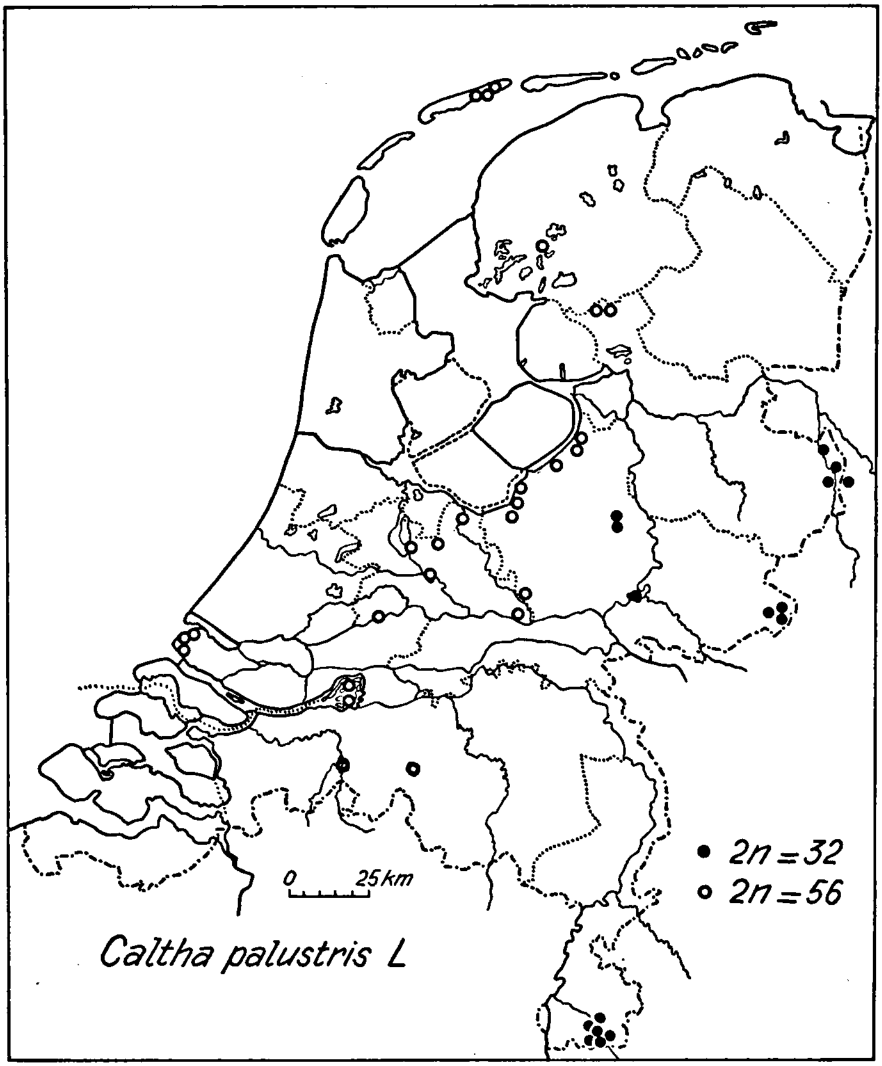

Fig. 5. The investigated populations of *C. palustris* in the Netherlands in 1965 and 1966.

ability is considerable. The leaf margins vary from entire to very coarsely serrate or dentate, and the shape of the leaves from reniform to deeply cordate; the flowers are large or small; the dorsal side of the carpels is straight or curved; erect but also prostrate plants occur. These are the differences on which the above-mentioned authors based their classification of the microspecies. In Reese's opinion the diagnoses of the microspecies as described by various authors are contradictory.

Till the present in our collections no obvious B-chromosomes could be demonstrated, contrary to Reese's observations in material originating from various sources in Germany.

Another interesting problem is the question of the basic chromosome number. In the literature this is generally considered to be $x = 8$. The number $2n = 56$ may in that case be regarded as heptaploid. KOOTIN SANWU (1965) is of the opinion that two basic numbers exist. According to him in Europe also diploid plants with $2n = 14$ should exist. LEONCINI (1951) describes a plant of *Caltha palustris* with the chromosome number $2n = 16$ originating from the botanical garden in Pisa. The existence of two basic numbers appears to be most plausible. The following euploid series are based on these basic numbers:

$x = 7$ 14, 28, 56

$x = 8$ 16, 32, 64.

The clearest morphological difference between the two cytotypes, $2n = 32$ and $2n = 56$, is the size of the pollengrains.

Cultivation experiments under the same conditions possibly may give valuable information with regard to the question whether the variation is due to environmental influences or whether it is genetically controlled.

The ecological preference of the two chromosome races proved also to be different. The $2n = 56$ plants probably prefer acid, not too oligotrophic soils, the $2n = 32$ plants grow in neutral or alcaline environments mostly richer in minerals. Measurements of the p.H. as well as other soil studies may give valuable information.

The author's preliminary conclusion is that in the Netherlands two cytotypes of *Caltha palustris* exist, with slight morphological differences and different ecological preferences.

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