TAXONOMICAL AND ECOLOGICAL STUDIES IN CALTHA PALUSTRIS L. II.

BY

PETRA G. SMIT

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I. INTRODUCTION

At least some of the species of the genus *Caltha* are polymorphic, showing not only a wide range of intraspecific morphological variation, but also considerable cytotaxonomic differentiation. A number of taxonomic problems are connected with this phenomenon.

In a previous paper (SMIT, 1967) a survey of the chromosome numbers of 46 Dutch populations was given. Two cytotypes of *Caltha palustris* were found, $2n=32$ and $2n=56$, respectively, with slight morphological differences and different ecological preferences.

WCISŁO (1964, 1967) studied *Caltha palustris* L. coll. in Poland, mainly from a cytotaxonomic viewpoint. She recognized three types which she regarded as subspecies rather than as species. Two of these, *C. palustris* L. ssp. *cornuta* S.N.K., $2n=32$, and ssp. *genuina* Hegi (=ssp. *palustris*), $2n=56$, were once found by her in the same population. In crossing experiments the author succeeded in obtaining hybrids between the cytotypes with the intermediate chromosome number $2n \doteq 44$.

With Wcisło's results in mind we may expect in the Netherlands at least two of the subspecies recognized by her, ssp. *cornuta* and ssp. *palustris*. Moreover, the degree of overlapping of these two subspecies proved to be so large that more studies in the whole area of this species complex seemed worth while. Therefore the study of *Caltha palustris* was continued with morphological methods, completed with biometric investigations, in order to search for possible differences between the cytotypes and to compare them with Wcisło's findings. The results are here presented, together with new counts of chromosomes, a preliminary survey of crossing experiments, and ecological data of populations in the Netherlands and Austria.

In the discussion some problems pertaining to the delimitation of the species *Caltha palustris* are dealt with.

II. MATERIAL AND METHODS

The plants studied were transferred from their natural habitat to the experimental garden.

The chromosome numbers were determined from roottip mitoses. To decrease the length of the chromosomes roottips of 2 cm, removed from potted plants or seedlings, received a pre-treatment of 4 hours in a 0.1 colchicine solution. Afterwards they were fixed in Carnoy, hydrolyzed in $_1$N HCl at 60 °C for 15 minutes, and stained with leucofuchsin (Feulgen), for one hour. The plastic coverslips of the preparations were removed in aceton one hour after squashing and replaced by glass coverslips that were mounted with Canada balsam.

The voucher specimens as well as the microscopical preparations are deposited in the Utrecht Botanical Museum and Herbarium.

The crosses were carried out with potted plants in the experimental garden. The emasculation of the flowers was performed in budstage. Before crossing, male and female flowerbuds were isolated with paperbags. When the flowers opened they were pollinated once a day during 4 consecutive days. The seeds were harvested 4–8 weeks after pollination. In order to attain the highest percentage of germination the seeds from the crossing experiments were sown soon after harvesting.

Seeds of *Caltha palustris* proved to have a poor germination rate. In connection with the crossing experiments it seemed necessary to investigate this problem further. Seeds collected in June 1966 and kept in dry storage since were set to germinate in Petri dishes on moist filter paper. Six samples of 50 seeds per dish of the cytotypes 2n = 32 and 2n = 56 were sown in the months of June, October, December, 1966, and February and April, 1967. The percentages of germinated seeds decreased in accordance with longer intervals between harvesting and sowing and became zero in April. The following results, equal for both cytotypes, were obtained: about 50 % of the seeds sown in June had germinated, 20 % of those sown in October, 15 % in December, 7 % in February and 0 % in April. It appears that *Caltha palustris* seeds only yield an optimal germination if the seeds are still green, and that their ability to germinate is almost entirely lost after 8 months, at least when they were stored dry. At present the author is attempting to find a better method for storing *Caltha* seeds.

III. RESULTS

III.1. *Chromosome numbers*

The chromosome numbers and the provenience of the plants are listed in table 1.

In some populations with 2n = 56 chromosomes plants rooting at the nodes were observed. REESE (1954) and WCISŁO (1967) found this kind of vegetative reproduction in tetraploids as well as in plants of higher polyploidy level. In the table the 2n = 56 plants which showed the phenomenon of rooting at the nodes are marked with an asterisk.

TABLE 1

A survey of the chromosome numbers of the investigated specimens, with reference to the collection number, number of records, number of studied plants, and the origin of the material. If not stated otherwise, the localities are in the Netherlands.

Coll. no.	Record	No. of plants studied	2n	Origin of the material
D700–708	67–1	7	32	Springwood near Middachten (Gelderl.).
D709–714	67–2	6	32	Wet forest near Middachten (Gelderl.).
D811–816	67–3	5	32	Springwood near Mook (Limburg).
D810	67–3	1	34	Springwood near Mook (Limburg).
D917–920	–	3	32	Austria: Hohe Tauern, alt. 850 m.
D926–930	67–16	5	32	Austria: Zitzmannsdorfer Wiesen, s.e. of Neusiedl am See.
D931–935	67–17	5	32	Austria: Auwald, on the bank of the river Leitha.
D936–940	67–18	4	32	Austria: wet pasture near Oberwart.
D941	67–19	1	32	Austria: wet Carex pasture near Hammerteich/Lockenhaus.
D942–945	67–20	4	32	Austria: Sphagnum peat near Hammerteich.
D946–948	67–21/22	3	32	Austria: pond near Güssing.
D949–953	67–23	4	32	Austria: Weineben near Deutschlandsberg, alt. 1500 m.
D954–955	–	2	32	Austria: Handalpe near Deutschlandsberg, alt. 1750 m.
D956–960	67–24	3	32	Austria: Hebalpe near Deutschlandsberg, alt. 1300 m.
D957	67–24	1	34	Austria: Hebalpe near Deutschlandsberg, alt. 1300 m.
D961–962	67–25	2	32	Austria: Hebalpe near Deutschlandsberg, alt. 1100 m.
D963–964	–	2	32	Austria: Lassnitzklause near Deutschlandsberg.
D965–966	67–26	2	32	Austria: Kresbach near Deutschlandsberg.
D985–993	–	4	32	Andorra: west of Soldeu, alt. 1750 m.
D994–995	–	2	32	Spain: Viella, alt. 900 m (Lérida).
D996	–	1	34	France: Hospice de France, alt. 1400 m (Dept. Ht. Garonne)
D997	–	1	36	France: Hospice de France, alt. 1400 m (Dept. Ht. Garonne).
D998–1005	–	4	32	Andorra: west of Soldeu, alt. 1750 m.
D1008–1011	–	2	32	Andorra: east of Soldeu, alt. 1700 m.
D715–719	–	2	56	Ditch south of Voorst (Gelderl.).
D852–856	67–6	3*	56	Pasture west of Emmen (Drente).
D857–861	67–7	3	56	Maden near Sleenerstroom (Drente).
D862–866	67–8	5*	56	Pasture near Oosterhesselen (Drente).
D867–871	67–9	4	56	Pasture between Westerbork and Elp (Drente).
D872–876	67–10	4*	56	Brook near Amelte (Drente).
D877–881	67–11	5	56	Wet pasture near Vries (Drente).
D882–886	67–12	3	56	Pasture between Donderen and Norg (Drente).
D887–891	67–13	3	56	Pasture between Dwingelo and Diever (Drente).
D892–896	67–14	4	56	Nieuwkoop, peatbog (S. Holland).
D464–473	–	3	56	Iceland: Engidalur.
D921–925	67–15	4	56	Germany: bank of the Chiemsee (Bavaria).
D976–979	–	3*	56	Ireland: bank of the river Camlin (S. Shannon).
D980–984	–	2*	56	Ireland: bank of Lough Ree, near Lanesborough (S. Shannon).
D1006–1007	–	2	56	Spain: Val d'Aran, alt. 1700 m. (Lérida).

III.2. *Crossing experiments*

a. Crosses were made between *Caltha palustris* plants with the chromosome number 2n = 32 and plants with 2n = 56.

One flower each of 12 different maternal plants of the cytotype 2n = 32 and of 16 maternal plants of the cytotype 2n = 56 was selfed. In only one selfing in the cytotype 2n = 56 viable seeds were obtained.

13 maternal plants of the cytotype 2n = 32 and 16 of 2n = 56 were pollinated (after emasculation) with pollen from a flower of the same inflorescence (geitonogamy). In this experiment no seeds were obtained. According to GALEN SMITH (1966, unpubl.) the plants are apparently self-incompatible.

Neither of the two cytotypes appears to be apogamous; 10 of the 2n = 32 flowers and 11 flowers of the cytotype 2n = 56 were emasculated but not pollinated. No seeds were formed.

Crosses with the cytotype 2n = 32 as maternal plant gave the following results:

22 crosses 2n = 32 ♀ × 2n = 32 ♂ yielded 19 plants with viable seeds and 3 plants (14 %) without seeds. The F_1 generation had 32 chromosomes.

19 crosses 2n = 32 ♀ × 2n = 56 ♂ yielded 10 plants with viable seeds and 9 plants (48 %) without seeds. Of the progeny of the 10 plants that had set seed 9 crosses had F_1 seedlings with 2n = 44 chromosomes and one with 2n = 32 chromosomes.

Crosses with the cytotype 2n = 56 as maternal plants gave the following results:

32 crosses 2n = 56 ♀ × 2n = 56 ♂ yielded 30 plants with viable seeds and 2 plants (6 %) without seeds. The F_1 generation had 2n = 56 chromosomes.

27 crosses 2n = 56 ♀ × 2n = 32 ♂ yielded 14 plants with seeds and 13 plants (48 %) without viable seeds. Of the progeny of the 14 plants that had set seed 12 had F_1 seedlings with 2n = 44 chromosomes and 2 with 2n = 56 chromosomes.

The 3 crosses which yielded an F_1 generation with the chromosome number of the motherplant and the selfing with viable seeds were probably due to insufficient precautions. The experiments were carried out in the garden, sometimes on days with much wind and in the neighbourhood of a large collection of *Caltha* plants.

In the crosses 2n = 32 × 32 and 56 × 56, 14 % and 6 %, respectively, did not set seed. In both the crosses 32 × 56 and the reciprocal ones 48 % of the plants failed to set seed. In the latter case the rate of seed-setting is significantly lower than in the first type of crosses. From these results we may conclude that there is only a partial incompatibility barrier between the two cytotypes.

The seeds obtained from the 32 × 32 and the 56 × 56 crosses germinated 3 weeks after sowing. The germination of the seeds of the 32 × 56 and the reciprocal crosses took more time, viz. at least 5 weeks.

In most of the crosses Dutch material was used, but some combinations

TABLE 2

Crosses between the cytotypes 2n = 32 and 2n = 56 from different European countries.

No. of cross	♀	2n	origin of the material	x	♂	2n	origin of the material	2n of the F_1
P 70	D176	32	Netherlands		D450	56	Austria	44
P 82	D448	56	Austria		D53	56	Netherlands	56
P 92	D346	56	Spain		D34	56	Netherlands	56
P 106	D393	56	Netherlands		D454	56	Austria	56
P 123	D336	56	Spain		D222	32	Netherlands	—
P 132	D249	56	Netherlands		D341	32	Spain	44
P 136	B163D	32	Finland		D338	56	Spain	44
P 143	D328	32	Spain		B163B	32	Finland	32

with other European *Caltha* plants were also made. Table 2 gives a survey of these crosses.

b. Cytotypes of *Caltha palustris* 2n = 32 and 56 were crossed with *Caltha palustris* cf. var. *alba* 2n = 44.

C. palustris cf. var. *alba* received as a cultivar from a Dutch nursery is a white-flowered plant with 44 chromosomes and pantoporate pollen grains. All previously studied pollen grains of *Caltha palustris* proved to be tricolpate.

12 maternal plants of *Caltha palustris* 2n = 32 and 23 maternal plants of the cytotype 2n = 56 were pollinated with pollen of *C. palustris* cf. var. *alba*; no seeds were obtained.

7 maternal flowers of *C. palustris* cf. var. *alba* were pollinated with *C. palustris* 2n = 32 and 7 flowers with *C. palustris* 2n = 56. Again no seeds were formed.

III. 3. *The correlation between morphological or physiological and cytological characters*

The correlation between the two chromosome numbers of *Caltha* in the Netherlands and some other characters, such as the time of flowering, the size of the pollen grains, the number of carpels, the diameter of the flowers, and the height of the plants was studied in populations that were cultivated for some years under uniform conditions in the experimental garden.

a. Time of flowering

In Poland a mixed population of two cytotypes, 2n = 32 and 56, had been found (WCISŁO, 1964). In this population the 2n = 32 plants were more precocious in flowering than the 2n = 56 plants. In general the lower polyploids proved to be more precocious than the higher polyploids and WCISŁO assumed that this could be one of the reasons for the absence of the cytotype 2n = 44 in nature.

Up till now in populations studied by the present author plants of only one cytotype were present, either 2n = 32 or 56. A comparison of flowering time of the two cytotypes in the same population was therefore impossible.

The time of flowering of the two cytotypes from different populations was studied in the experimental garden. Every week in 1966 and 1967, from the beginning till the end of the flowering period, in 13 populations of *C. palustris* 2n = 56 and 11 populations 2n = 32, the date of opening of the first flower and the flowering date of 50 % of the plants of a population were noted.

In 1967 spring came earlier than in 1966, and most of the populations flowered 14 days earlier in that year.

The data assembled in figure 1 demonstrate a large range of variation for the populations of both cytotypes. Yet nothing indicates that the lower polyploids are more precocious than the higher ones. Field observations gave the same impression, but could not be worked out quantitatively, as it was impracticable to visit these populations every week.

In their natural habitat as well as in cultivation the plants from the isle of Terschelling (2n = 56) and from springwoods in Limburg (2n = 32) flowered later than other populations of the two cytotypes. The phenotypic variation in the time of flowering of *C. palustris* which varies in the different

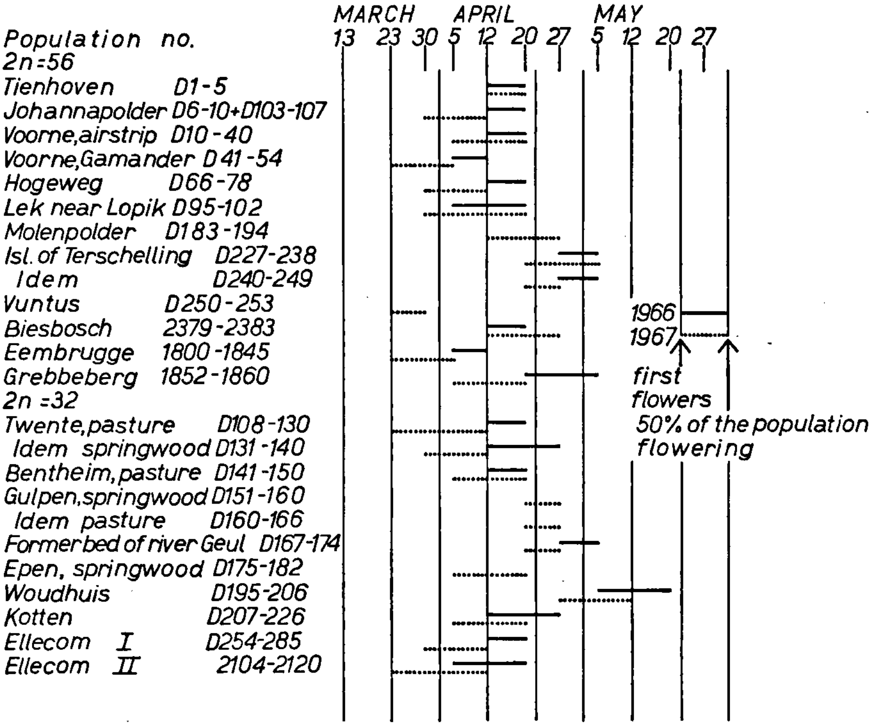

Fig. 1. Survey of the variation of the time of flowering in 1966 and 1967 of plants of 13 2n = 56 and 11 2n = 32 populations of *C. palustris* cultivated in the experimental garden.

parts of its area may have a genotypic basis. This variation may be the result of the following circumstances. The isle of Terschelling in the North of the Netherlands always has a late spring, for during the winter the seawater cools considerably and in spring for a long time influences the air temperature on the island adversely. The plants from springwoods in Limburg in the South of the Netherlands also flowered late, although spring always comes early in Limburg. However, the water temperature of springwoods fluctuates little in the course of the year and is rather low during the vegetation period of *Caltha*.

b. The pollen grains

In plants of both cytotypes, taken at random from 5 different populations, the diameter of the pollen grains of the plants in the wild were compared with cultivated for two years plants of the same populations. There proved to be virtually no difference in the size of the pollen grains between wild plants and the same plants under cultivation.

The results of 2500 measurements of pollen grains of plants belonging to 25 populations are shown in figure 2.

The standard error of difference between the two cytotypes is 0.08. The observed difference between the diameter of the cytotypes $2n=32$ and $2n=56$ is 4.33 μ. This is much more than twice the standard error of difference (0.16) and it is probably well-established that the cytotypes with $2n=56$ chromosomes have larger pollen grains than the $2n=32$ cytotypes.

NOTE: In the first publication by the present author there was an error in the calculation of the standard deviations of the pollen grains; the figures should have been multiplied by the testing number 3.9 for the ocular micrometer used (SMIT, 1967: 505).

c. Number of carpels, diameter of the flowers, and height of the plants.

Table 3 gives a survey of these three characters from 1965 to 1967. In 1965 the measurements were taken from plants in the wild out of 8 $2n=32$ and 9 $2n=56$ populations, in the years 1966 and 1967 with material from the same populations of both cytotypes but cultivated in the experimental garden.

The number of the carpels remained fairly constant during these three years.

Unfortunately the diameter of the flowers was not measured in 1966. The difference between the two cytotypes in this character was larger in 1967 in the cultivated plants, as compared with the wild plants in 1965. The $2n=32$ flowers became smaller and the $2n=56$ ones larger in the two years of cultivation.

The height of the plants of both cytotypes decreased during cultivation.

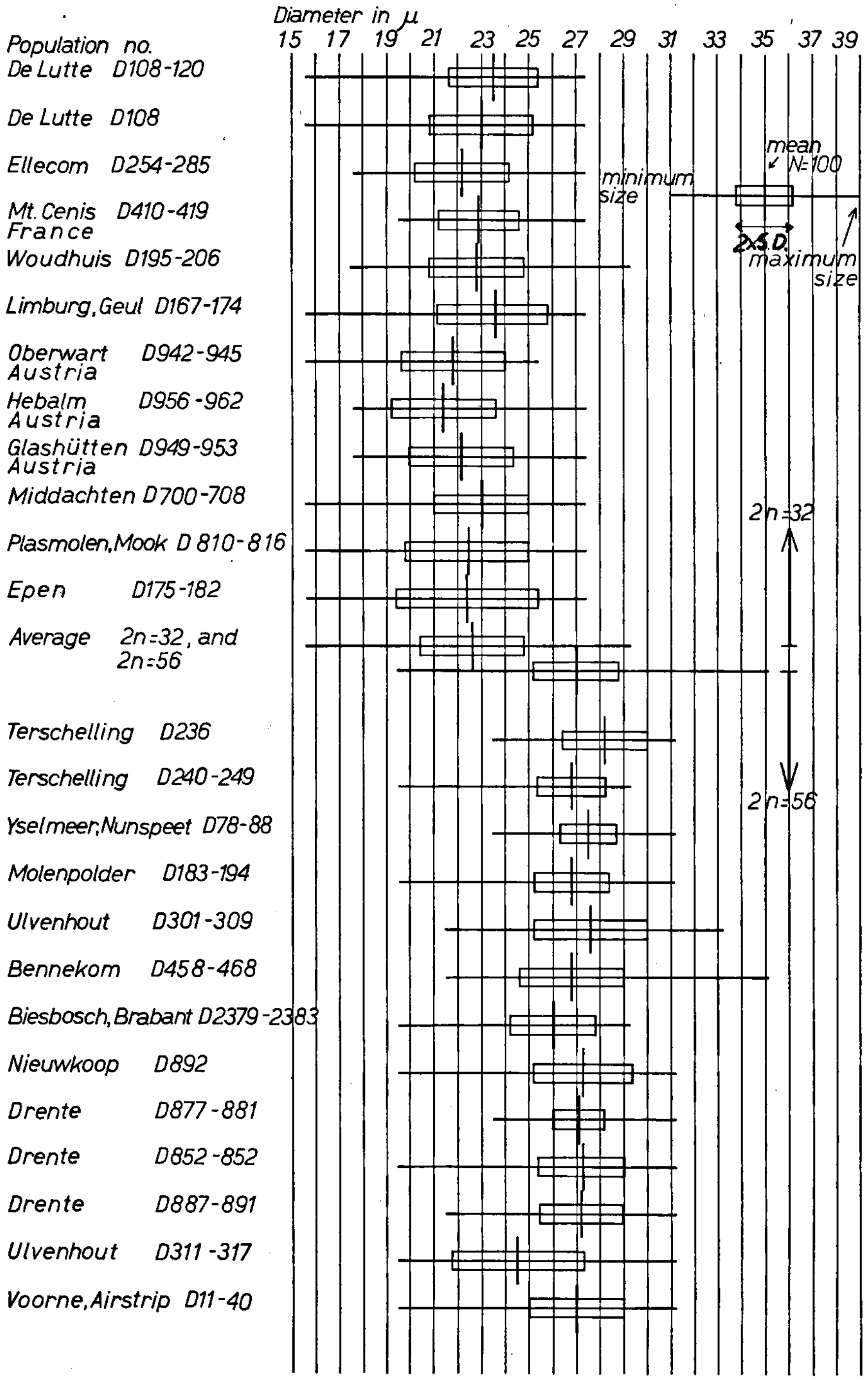

Fig. 2. Ranges of variation in the diameter of the pollen grains in 25 populations of *C. palustris* in the wild. The horizontal lines represent the ranges, the vertical lines the means, and the rectangles twice the standard deviation from the mean. In this case the standard error (S.E.) of the mean is 1/10 of the standard deviation (S.D.), for N=100 and $S.E. = \sqrt{(S.D.^2)/N} = S.D./\sqrt{N}$, too small to indicate in this figure.

TABLE 4: Records of vegetations with *Caltha palustris*, on the right 2n=32, on the left 2n=56.

No. of record	2n=56 Presence of 28 records	2n=32 Presence of 21 records	14	10	6	15	11	7	8	9	18	19	25	21	22	16	17	20	3	2	1	26
Date in 1967			28–4	24–4	24–4	26–5	24–4	24–4	24–4	24–4	2–6	3–6	6–6	3–6	3–6	29–5	31–5	3–6	13–4	22–3	22–3	7–6
Surface (sq.m.)			16	2	15	16	16	9	16	16	25	200	40	5	25	25	10	15	25	40	25	12
Watersurface (%)			—	—	—	15	10	—	—	—	15	—	—	90	70	—	—	60	35	—	30	—
Layer of herbs, covering (%)			90	85	80	100	95	95	95	95	100	100	90	100	90	100	100	80	35	80	70	100
Layer of herbs, height (cm)			40	30	30	50	30	30	25	30	60	80	30	100	120	100	140	80	60	20	20	80
Layer of trees, covering (%)			—	10	—	—	—	—	—	—	—	20	45	—	10	—	—	60	10	30	15	80
Layer of trees, height (m)			—	8	—	—	—	—	—	—	—	2	2,5	—	2	—	—	5	18	20	7	20
Layer of mosses (%)			30	—	55	70	5	—	50	—	20	—	20	—	—	40	—	—	10	—	—	—
No. of species not listed			1	1	2	6	0	1	3	1	8	2	6	0	1	4	3	5	6	3	2	6
									2n=56		2n=32											
CALTHA PALUSTRIS	28	21	3.2	+.2	+.2	2.2	2.2	1.2	2.2	2.2	3.2	+.1	3.2	2.2	1.2	2.2	3.2	3.2	1.2	2.2	3.2	4.2
Species of the Ord. *Phragmitetalia*																						
Glyceria maxima	10	0	—	2.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glyceria fluitans	6	4	—	—	1.2	—	3.2	1.2	2.2	—	—	—	—	—	—	—	—	—	—	—	—	—
Phragmites communis	12	1	3.2	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—
Iris pseudacorus	5	6	—	—	—	—	—	—	—	—	—	—	—	1.2	1.2	+.1	1.2	—	—	—	—	—
Ord. *Magnocaricetalia*																						
Equisetum fluviatile	7	2	—	—	1.1	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carex paniculata	2	2	1.2	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—
Carex acuta	4	5	—	—	—	—	—	—	—	—	—	3.2	—	—	3.2	—	—	2.2	—	—	—	—
Carex riparia	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	2.2	—	—	—
Carex acutiformis	1	1	—	—	—	3.2	—	—	—	—	—	—	—	—	—	4.3	—	—	—	—	—	—
Species of the Ord. *Caricetalia fuscae*																						
Carex echinata	0	1	—	—	—	—	—	—	—	—	3.2	—	—	—	—	—	—	—	—	—	—	—
Carex nigra	2	3	—	—	—	+.2	—	—	2.2	—	1.2	2.2	+.2	—	—	—	—	—	—	—	—	—
Ranunculus flammula	5	0	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—
Comarum palustre	1	0	—	—	—	2.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Species of the Class *Molinieto-Arrhenatheretea*																						
Holcus lanatus	11	4	—	3.2	3.2	—	3.2	2.2	2.2	3.2	2.2	+.1	—	—	—	—	—	—	—	—	—	—
Rhytidiadelphus squarrosus	2	0	—	—	—	—	—	—	3.2	—	—	—	—	—	—	—	—	—	—	—	—	—
Cardamine pratensis ssp. pal.	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—
Rumex acetosa	11	3	+.1	+.1	3.1	—	—	3.2	+.1	1.1	+.1	—	—	—	—	—	—	—	—	—	—	—
Anthoxanthum odoratum	5	3	—	—	—	—	—	—	—	2.2	2.1	—	1.2	—	—	—	—	—	—	—	—	—
Cerastium holosteoides	8	1	—	—	—	—	1.2	+.2	+.1	1.2	—	—	—	—	—	—	—	—	—	—	—	—
Trifolium pratense	2	1	—	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—
All. *Arrhenatherion*																						
Cardamine pratensis ssp. prat.	20	8	+.1	+.1	+.1	+.1	1.2	2.2	2.2	1.1	+.1	—	—	—	+.1	—	—	—	—	+.2	2.2	—
Ranunculus acer	13	5	—	—	—	—	—	1.2	2.2	2.2	1.2	—	—	—	—	—	—	—	—	+.1	—	—
Festuca pratensis	1	2	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—
Bellis perennis	6	0	—	—	—	—	+.1	1.2	—	2.2	—	—	—	—	—	—	—	—	—	—	—	—
Trifolium repens	4	2	—	—	—	—	+.2	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—
All. *Molinion coeruleae*																						
Thalictrum flavum	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—	—	—	—
Achillea ptarmica	3	2	—	—	—	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sanguisorba officinalis	0	2	—	—	—	—	—	—	—	—	+.1	1.1	—	—	—	—	—	—	—	—	—	—
Juncus effusus	5	7	—	—	—	+.2	+.1	—	2.2	—	2.2	—	3.2	1.2	—	—	—	—	—	—	—	—
Cirsium palustre	2	3	—	—	—	—	—	—	+.1	—	—	—	+.1	—	—	—	—	—	—	—	+.1	—
Lychnis flos-cuculi	10	6	—	1.2	—	1.1	+.2	1.2	1.2	1.2	2.2	+.1	1.2	—	+.1	—	—	—	—	—	—	—
Lysimachia vulgaris	3	5	+.2	—	—	—	—	—	—	—	—	—	—	—	1.1	+.1	1.2	—	—	—	—	—
Juncus conglomeratus	0	2	—	—	—	—	—	—	—	—	—	4.2	—	—	—	—	—	1.2	—	—	—	—
Valeriana dioica	0	5	—	—	—	—	—	—	—	—	1.1	—	3.1	11.	1.1	1.1	—	—	—	—	—	—
Equisetum palustre	7	9	—	—	—	+.1	—	—	—	—	2.1	—	—	1.2	3.1	—	—	2.1	—	—	+.1	—
Myosotis palustris	8	8	—	—	—	—	—	—	—	—	2.1	1.1	—	1.1	—	—	—	—	—	+.2	—	—
Galium palustre	13	11	2.2	2.2	—	—	—	—	+.1	—	1.1	1.2	1.2	2.1	2.2	1.2	—	+.1	+.1	1.1	1.1	—
Mentha aquatica	8	11	—	—	—	—	—	—	+.1	—	—	—	1.1	—	—	1.1	2.1	—	+.1	1.1	1.1	—
Ass. *Cirsieto-Molinietum*																						
Cirsium dissectum	1	3	—	—	—	—	—	—	—	—	1.1	1.1	—	1.1	—	—	—	—	—	—	—	—
Carex hostiana	2	1	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—
Carex panicea	2	3	—	—	—	3.2	—	—	—	—	+.1	—	1.2	—	—	1.2	—	—	—	—	—	—
Agrostis canina	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—
All. *Filipendulion*																						
Filipendula ulmaria	10	14	3.2	1.2	—	2.2	1.2	—	—	—	—	1.1	—	4.3	—	—	—	1.2	2.2	+.1	1.1	—
Angelica sylvestris	0	7	—	—	—	—	—	—	—	—	1.1	—	—	1.1	—	—	—	—	—	—	+.1	1.1
Valeriana officinalis	5	5	2.1	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1
Scirpus sylvaticus	1	7	—	—	—	—	—	—	—	—	1.1	—	3.1	2.2	3.2	—	—	1.2	—	—	—	—
Lythrum salicaria	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	—	—	—	—	—
Epilobium hirsutum	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—
Phalaris arundinacea	7	2	—	1.2	—	—	1.2	—	—	1.2	—	—	—	—	—	—	4.3	—	—	—	—	—
Species of the Ord. *Fagetalia*, All. *Alno-Padion*																						
Symphytum officinale	6	2	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	2.2	—	—	—	—	—
Glechoma hederacea	5	2	—	—	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—	—
Deschampsia caespitosa	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.3	—	—
Urtica dioica	2	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	1.2
Ranunculus ficaria	2	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.1	—
Salix spec.	1	7	—	—	—	—	—	—	—	—	—	—	3.2	—	1.2	—	—	3.3	—	—	1.2	—
Alnus glutinosa	3	15	—	1.1	—	—	—	—	—	—	—	2.1	2.2	—	—	—	—	3.2	1.2	2.1	3.2	4.1
Poa palustris	0	1	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—
Equisetum telmateia	0	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—
Cardamine amara	1	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	2.2
Chrysosplenium oppositifolium	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	—	4.3	—
Primula elatior	0	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—
Anemone nemorosa	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.2	—	1.1	—	—
Ajuga reptans	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+.2	—	1.1	—	—
Species of the All. *Agropyro-Rumicion crispi*																						
Poa pratensis	12	0	2.2	1.2	1.2	—	+.1	2.2	1.2	2.2	—	—	—	—	—	—	—	—	—	—	—	—
Poa trivialis	5	9	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	2.2	—	—	—	—	1.2
Ranunculus repens	9	10	—	1.2	1.2	1.2	2.2	2.2	3.1	3.2	2.1	1.2	2.2	1.1	1.1	—	+.1	+.1	1.2	—	—	2.2
Lysimachia nummularia	5	6	—	—	—	1.2	1.2	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—
Acc. *species*																						
Calamagrostis canescens	3	0	+.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Taraxacum officinale	7	1	—	1.1	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 3

Survey of the number of carpels in each flower, the flower diameter, and the height of the fruit-bearing plants of 8 *Caltha* populations 2n=32 and 9 populations with plants with 56 chromosomes, under cultivation for three years.

		$\bar{x}_{32}$	S.D.	S.E.	$\bar{x}_{56}$	S.D.	S.E.	$\bar{x}_{56}-\bar{x}_{32}$	2× S.E. of D.	N_{32}	N_{56}
No. of carpels	1965	8,99	2,63	0,42	12,24	4,70	0,74	3,25	1,16	40	40
	1966	9,36	2,73	0,18	12,56	3,15	0,28	3,20	0,66	236	126
	1967	9,97	2,60	0,16	13,21	3,68	0,28	3,24	0,64	257	177
Diam. of the flowers (mm.)	1965	36,13	7,02	0,79	40,61	6,17	0,69	4,48	2,06	80	80
	1967	32,52	4,83	0,54	43,21	5,66	0,86	10,69	2,02	79	43
Height of the plants (cm.)	1965	28,83	13,88	2,20	38,12	17,91	2,83	9,29	7,62	40	40
	1966	22,54	4,74	0,60	26,14	7,38	1,11	3,60	2,50	63	44
	1967	18,22	4,52	0,53	24,06	6,73	0,97	5,84	2,20	73	48

In the first year, 1966, it was more than in the second year. However, the ratio between the average height of the plants with 32 chromosomes and that of the 2n = 56 plants remained about 3 : 4.

In 1967 the resemblance between the ratios of averages of a character of the two cytotypes was remarkable. In three different characters the ratio always turned out to be 3 : 4.

III.4. *The relation between cytological characters, geographical distribution, and ecological preference*

In the course of the present studies *Caltha palustris* plants with the chromosome numbers 2n = 32 and 2n = 56 had been met with in the Netherlands. In the collections from Austria, Spain, France, Germany, Ireland, and Iceland usually these two cytotypes were also found.

Table 4 gives the phytosociological analysis of vegetations with *Caltha palustris*, made in 1967 in the Netherlands and in Austria, with on the right the records of plants with the chromosome number 2n = 32 and on the left those with 2n = 56. There are also two columns for the presence of the species in the records as a whole, also including the records published in Smit (1967). This table is somewhat differently arranged in comparison with table 5 in Smit (1967). The use of the name *Filipendulo-Petasition* is avoided, because this alliance probably is not a natural entity but should be divided into a *Filipendulion* in a restricted sense and another alliance (Segal, 1966). The alliance *Alno-Ulmion* did not seem to be a good choice; some of the species listed in this alliance are now placed in the *Agropyro-Rumicion crispi*, an alliance of more or less natural plant communities with important anthropogenic influences. The presence of the species in this alliance is equal in the records of both cytotypes.

VAN DE BROEK and DIEMONT (1966) described the subassociation *Querceto-Carpinetum filipenduletosum* in Limburg, in the South of the Netherlands, as being an extremely moist form of the *Querceto-Carpinetum* association. This subassociation differs from other subassociations by the presence of species preferring a very moist milieu and partly by shade-tolerant swamp plants like *Angelica sylvestris, Alnus glutinosa, Filipendula ulmaria, Cardamine pratensis, Carex acutiformis, Crepis paludosa, Lysimachia vulgaris, Equisetum telmateia,* and *Caltha palustris.* A part of the marshy forest vegetations formely classed in the *Querceto-Carpinetum filipenduletosum,* viz. the part that contains no or only a few *Carpinion betulus* species, can better be classed in the *Pruneto-Fraxinetum* of the *Alno-Padion*; this applied to the records listed in table 4.

In the records with the cytotype 2n = 32 the presence of the *Alno-Padion* species is high: ± 22 %, four times as much as in the records with the cytotype 2n = 56. On the other hand, in the records with 2n = 56 *Caltha* plants the presence of species of the *Phragmitetalia, Magnocaricetalia,* and *Caricetalia fuscae* orders is higher.

The presence of the species of the *Molinieto-Arrhenatheretea* class is over 50 % for the total number of records of both cytotypes. Various authors disagree with regard to the synecological subdivision of this class. In the recent literature *Caltha* is also reported as a characteristic species of the *Calthion* (WESTHOFF, 1966, mimeographed manuscript).

In the records with the cytotype 2n = 32 *Caltha* grows in vegetations in which species from the alliances *Filipendulion* and *Molinion coeruleae* occur regularly. In records with the cytotype 2n = 56 there is a higher degree of presence of species of the *Arrhenatherion* and also the *Molinion alliance.* The *Calthion* and the *Filipendulion,* as described by WESTHOFF, belong to the *Molinietalia* order, with for the *Calthion* among others the characteristic species *Caltha palustris, Lychnis flos-cuculi,* and *Scirpus sylvaticus.* In the total number of records (49) the presence of these *Calthion* species (except for *Caltha,* because a record was only made if *Caltha* was present) was low. For practical reasons, e.g., for spotting ecological differences between the two cytotypes, it seemed preferable to use an older classification of the *Molinio-Arrhenatheretea.*

Caltha was strikingly absent from the springwoods of the Renkumse beek, the Heelsumse beek, Sonsbeek, and Beekhuizen in the vicinity of Arnhem. The springwater rises here in pure preglacial sands (MAAS, 1959), has a lower pH, and contains fewer minerals than that of the springwoods where *Caltha* 2n = 32 occurs, which is neutral to alcaline and contains, i.a., more Ca and Mg ions.

IV DISCUSSION

Up till now 56 populations in the Netherlands and 36 from other European countries have been investigated from a cytological and for the greater part also from an ecological point of view.

According to Reese (1954) different chromosome numbers in *Caltha palustris s.l.* show no correlation with the taxonomic subdivision into species, subspecies, and formae proposed by earlier authors.

The taxonomic value of cytological data varies from one group to another. When the infraspecific taxonomy of *Caltha palustris* in the Netherlands is based on the presence of two different cytotypes, $2n=32$ and $2n=56$, the species can be said to comprise two cytotypes with slight morphological differences and different ecological preferences which are compatible in crosses, but these differences are insufficient for distinguishing separate subspecies or even species.

On the basis of these cytological, ecological, and morphological studies in populations.of *Caltha palustris s.l.* the Dutch plants can be placed in the species *Caltha palustris*, with series of more or less discontinuous ecotypes with associated morphological differences. The word ecotype is applied here as defined by Gregor, Davey and Lang (1936): "A population distinguished by morphological and physiological characters, most frequently of a quantitative nature; interfertile with other ecotypes of the ecospecies, but prevented from freely exchanging genes by ecological barriers". The transition between the ecotypes with the chromosome number $2n=32$ as compared with those with $2n=56$ chromosomes are only by degrees more discontinuous than in the different ecotypes of the same chromosome number.

When the two Irish populations, which agree with Forster's description (1807) of *Caltha radicans*, are compared with the Dutch material of Ulvenhout, all small plants with long-creeping stems rooting at the nodes, no cytological or morphological differences were evident. But between these Dutch populations and other populations of the same cytotype ($2n=56$) there were continuous transitions, i.e., between so-called *C. radicans* and *C. palustris*. Under comparative experimental cultivation the length of the stems rooting at the nodes decreased to 20 cm, whereas the stems of these plants reached a length of 1 m in their natural habitat at Ulvenhout. *C. "radicans"* is quite prostrate. Some populations in Drente with more erect plants also contain plants rooting at the nodes, but in their natural habitat their stems are much shorter. The very tall but quite erect plants of populations in the Biesbosch also rooted at the nodes, but never produced new plants; the upright stems dried out after flowering, together with the young plants formed at their nodes. As shown by all this evidence, and by studies of 800 herbarium sheets, the two forms are not sufficiently distinct to be treated as distinct species or subspecies. The plants of *Caltha* with creeping stems and rooting nodes, the exact nature of which is not understood, seem local facies of the species, and it seems better to treat them as a variety of *Caltha palustris*: *C. palustris* L. var. *radicans* (Forst.) Beck (1886).

After having studied a series of plants of *Caltha palustris* L. var. *arctica* (R. Br.) Huth from the herbarium in Washington (US) from Alaska, e.g., Spetzman 2392 Point Barrow; Thomson 1354 Lake Noluk; Ward 1488 East Oumalik; Gillis 1088 Jago River; Campbell s.n. St. Lawrence Isl.; Mertie 177 Nushagak; Hagelbarger 248 Katmai; and the description of R. BROWN in Parry, 1th Voy. App.: 265 (1823), the author arrived at the conclusion that *Caltha palustris* var. *arctica* (R. BR.) HUTH is identical with *Caltha palustris* var. *radicans* (FORST.) BECK.

In a publication of 1967 WCISŁO gave the following subdivision of *C. palustris*: *Caltha palustris* L. ssp. *cornuta* S.N.K., ssp. *genuina* Hegi (=ssp. *palustris*), and ssp. *laeta* S.N.K. This is not acceptable for the present author. The delimitation of *C. palustris* in Hegi's flora was based on the work of SCHOTT, NYMAN and KOTSCHY, and BECK. Unfortunately the original collections of the three first-named authors were destroyed in the war. But these authors' material and also that of Beck was from the same parts of East Europe, and Beck probably studied their collections. In Beck's East European material, kindly sent on loan from the Prague Herbarium (PRC), again continuous series could be observed, and it seemed impossible to divide it in the taxa distinguished by other authors in eastern Europe, e.g. by WCISŁO.

Plants of *Caltha* in populations studied by the present author in the vicinity of Deutschlandsberg (Austria) agreed more or less with the subspecies *laeta*, said to be a montane subspecies. At an altitude of 1650 m these plants were quite small, up to 15 cm high, growing in an open marshy vegetation above. the local timberline. Lower (1300–1100 m) on the same mountain *Caltha* plants grew on the banks of small rivulets in coniferous forest; here they were taller, up to 30 cm, and only 200 m lower, at 900 m, they reached 60–80 cm. In this area further plants were observed on the banks of a small river in an *Alno-Padion* forest. In these three populations consisting of plants with 32 chromosomes the leaf-margins were as a rule distinctly crenate, and the follicles had straight dorsal sides, both said to be characters of ssp. *laeta*. In the population at 1300–1100 m, beside follicles with straight dorsal sides also follicles with concave dorsal sides were present, a distinctive character reported for ssp. *cornuta*; the plants that bore them had, however, distinctly crenate or dentate leaf-margins, an alleged character of ssp. *laeta*. In the population at 900 m the leaves were distinctly crenate or dentate, but the angular divergence between the basal lobes of the leaves was 90° (ssp. *laeta* is stated to have an angular divergence of 30°), i.e., the margins of the leaves were typical for ssp. *laeta*, the angular divergences for ssp. *cornuta*.

"The subspecies, as a considerable segment of a species with a distinct area and more or less distinct morphology, often showing some intergradation, clearly fulfils a useful purpose" (DAVIS and HEYWOOD, 1963); it is on a higher taxonomic level than the infraspecifically distinct types met with in our material.

These considerations and the results of crossing experiments lead us to the conclusion that *Caltha palustris* is a species with large genecological differentiation in its populations. The intraspecific polyploidy, which could have arisen by mutations, would have enhanced the polymorphism of the species, resulting in series of more or less discontinuous ecotypes with associated morphological characteristics.

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