

# CYTOTAXONOMIC STUDIES IN THE GENUS SYMPHYTUM

## I. SYMPHYTUM OFFICINALE L. IN THE NETHERLANDS

BY

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### INTRODUCTION

The genus *Symphytum* contains about 25 species (BUCKNALL, 1913). In the Netherlands it is represented by one indigenous species, *S. officinale* L.; two others, *S. asperum* Lepech. and *S. bulbosum* Schimp., have been introduced but have not become established.

*S. officinale* is a variable species. KERNER (1863) described a segregate, *S. uliginosum*, which was reduced to subspecific rank by NYMAN (1878): *S. officinale* L. ssp. *uliginosum* (Kern.) Nym. The differences between subspecies *officinale* and *uliginosum* are: in ssp. *officinale* the stems and leaves are more or less densely hispid, in ssp. *uliginosum* they are provided with small white deciduous prickles with tubercular base. Furthermore in the first-mentioned ssp. the upper stem-leaves are entirely decurrent but in the last named they are not or partially decurrent. There is also a difference in the colour of the flowers: white, yellowish, or dark purple in ssp. *officinale*, and red-purple or violet in ssp. *uliginosum*.

*S. asperum*, which occurs in the Caucasus, Armenia, and Persia hybridizes with *S. officinale* (LINDMAN, 1911; BUCKNALL, 1912; FAEGRI, 1931; TUTIN, 1956; WADE, 1958). This hybrid, *S. x uplandicum* Nym., has also been recorded from the Netherlands (DE WEVER, 1932; KLOOS, 1934; VAN OOSTSTROOM and REICHGELT, 1961), but it is apparently not very common.

In England the populations of hybrids are remarkably uniform, although great variations can be observed. Backcrossing with *S. officinale* may result in highly variable hybrid-swarms (WADE, 1958). Neither in England nor in the Netherlands it is likely that backcrossing in the direction of *S. asperum* occurs, in view of the extreme rarity of this species.

In Western Europe *S. officinale* is most often characterized by white flowers, (TUTIN, 1956), whereas no white-flowering individuals have been reported from Russia, (STEVEN, 1851; POPOV, 1953). According to TUTIN (l.c.) it is tempting to suggest that the purple-flowered form may be due to introgression between *S. officinale* and *S. asperum*.

In Britain *S. x uplandicum* is probably the commonest *Symphytum*

(TUTIN, l.c.). WADE (1958) is of the opinion that originally most of the hybrids were introduced into Britain from Russia as fodderplants. The hybrids have purple flowers (TUTIN, l.c.).

According to the literature cytological studies in *Symphytum officinale* and *Symphytum asperum* yielded the following results:

**Symphytum officinale:**  $2n = 36$  (STREY, 1931; cult. mat.);  $2n = 42$  (DATTA, 1933; cult. mat.);  $2n = 48$  (TARNAVSCHI, 1948; Roumania);  $2n = \pm 40$  (LÖVE and LÖVE, 1956; Iceland);  $2n = 26, 40, 48$  (GADELLA and KLIPHUIS, 1963, 1967; the Netherlands).

**Symphytum asperum:**  $2n = \pm 36$  (STREY, 1931; cult. mat.);  $2n = 40$  (BRITTON, 1951; cult. mat.).

The number  $2n = 26$ , reported by GADELLA and KLIPHUIS (1963) was counted in a single plant collected in an osier-bed, near IJsselstein (prov. of Utrecht). In 1964 and 1965 large numbers of plants were collected there. It was found that a number of plants was characterized by the number  $2n = 24$ , whereas other individuals had the number  $2n = 24 + 1-4 B$ .

The basic number of *S. officinale* seems to be  $X = 12$ . The number  $2n = 40$  does not fit into the euploid series.

In view of the fact that various chromosome numbers have been reported for *S. officinale* and *S. asperum*, it seemed indicated to undertake combined cytological and morphological studies, supplemented by field-studies and experimental cultivation. By crossing experiments it may be possible to resynthesize the hybrid *S. x uplandicum* and to determine its chromosome number. If back-crossing of this hybrid occurs in the Netherlands, it would be interesting to repeat such crossings under experimental conditions and to study any produced hybrids, both morphologically and cytologically.

In this paper a survey is given of the chromosome numbers of plants from 72 different populations in the Netherlands, together with some morphological data. The results of biometric studies in different populations, in the wild, as well as under controlled conditions, and crossing experiments between the three Dutch cytotypes and material from elsewhere will be published later.

#### MATERIAL AND METHODS

Seventy-two populations of *Symphytum officinale* were the object of these studies. From each population 1-20 plants were dug out, depending on the number of plants available and the degree of variation of the population. The plants were transplanted to the Botanical Garden where they were grown in pots.

The determinations of the chromosome numbers were carried out from roottip-mitoses. Sometimes the fixations were made directly in the field, but in most cases the roottips were removed from potted plants. The cytologically studied plants were transferred to the experimental plot.

The roottips were fixed in Karpechenko's fixative, embedded in paraffin, sectioned at 15 micron and stained according to Heidenhain's haematoxylin method. Voucher specimens, collected both in nature and after 1-3 years of cultivation, as well as the microscopical preparations have been deposited in the Botanical Museum and Herbarium of the State University of Utrecht.

## RESULTS

In this chapter a survey of the cytological results is given, as well as some preliminary notes on crossing experiments and on morphological differences in the various populations.

### I. Cytological data

511 plants, originating from 72 different sources were studied cytologically. The table gives a survey of the chromosome numbers, together with the collection numbers and places of origin of the material. Some short notes on edaphic factors have been added.

TABLE

A survey of the chromosome numbers of the investigated plants, with reference to the collection numbers and the place of origin of the material.

pop. 1	In an osier-bed near IJsselstein (prov. of Utrecht), on river clay*)	
	coll. no.: G. et K. 530 (1962) . . . . .	2n = 24
	coll. no.: G. et K. 1248-1251 (1964) . . . . .	2n = 24
	coll. no.: G. et K. 1544-1554 (1964) . . . . .	2n = 24
	coll. no.: G. et K. 1568-1584 (1964) . . . . .	2n = 24
	coll. no.: G. et K. 3116-3118 (1965) . . . . .	2n = 24
pop. 2	In an osier-bed of the estate "Oude-Gein" near Jutphaas (prov. of Utrecht), on river clay	
	coll. no.: G. et K. 3129-3133 (1965) . . . . .	2n = 24
pop. 3	In an osier-bed between IJsselstein and Jutphaas (prov. of Utrecht), on river clay	
	coll. no.: G. et K. 3124-3127 (1965) . . . . .	2n = 24
	coll. no.: G. et K. 3128 (1965) . . . . .	2n = 48
pop. 4	On a refuse dump near IJsselstein (prov. of Utrecht), on river clay	
	coll. no.: G. et K. 4149 (1966) . . . . .	2n = 24
	coll. no.: G. et K. 4139-4148 (1966) . . . . .	2n = 48
pop. 5	In an orchard near IJsselstein (prov. of Utrecht), on river clay	
	coll. no.: G. et K. 4151, 4152 (1966) . . . . .	2n = 24
	coll. no.: G. et K. 4150, 4153 (1966) . . . . .	2n = 48
pop. 6	At the border of the Sneeker Meer near Goingsarijp (prov. of Friesland), on peaty clay	
	coll. no.: G. et K. 4052 (1966) . . . . .	2n = 40

\*) In various plants of this population B-chromosomes (1-4) were found.

pop. 7	At the border of "Tienensloot", between Uitwellingergra and Goingaripp (prov. of Friesland), on peaty clay	
	coll. no.: G. et K. 5693-5697 (1966)	2n = 40
	coll. no.: G. et K. 5700-5707 (1966)	2n = 40
	coll. no.: G. et K. 5710-5713 (1966)	2n = 40
pop. 8	At the border of "Wijde Wijmers", between IJlst and Heeg (prov. of Friesland), on peaty clay	
	coll. no.: G. et K. 5691-5692; 5709 (1966)	2n = 40
pop. 9	At the border of "Jeltesloot", between Heeg and Langweer (prov. of Friesland), on peaty clay	
	coll. no.: G. et K. 5698-5699 (1966)	2n = 40
pop. 10	At the border of a ditch between Elburg and Kampenieuwstad (prov. of Gelderland), on peaty clay	
	coll. no.: G. et K. 1172-1177 (1964)	2n = 40
	coll. no.: G. et K. 2214-2223 (1965)	2n = 40
	coll. no.: G. et K. 4562-4567 (1966)	2n = 40
pop. 11	Hoophuizen near Harderwijk (prov. of Gelderland), at the border of a ditch, on peaty clay	
	coll. no.: G. et K. 1178-1181 (1964)	2n = 40
pop. 12	2 km W. of Ermelo (prov. of Gelderland), at the border of a ditch on moist, loamy sand soil	
	coll. no.: G. et K. 2183-2192 (1965)	2n = 40
	coll. no.: G. et K. 4558-4561 (1966)	2n = 40
pop. 13	On a "legakker" *) in the Molenpolder near Westbroek (prov. of Utrecht), on peaty clay	
	coll. no.: G. et K. 338 (1962)	2n = 40
	coll. no.: G. et K. 4854-4856 (1966)	2n = 40
pop. 14	At the border of a lake near Oud-Loosdrecht (prov. of Utrecht), on peaty clay	
	coll. no.: G. et K. 1342-1343 (1964)	2n = 40
	coll. no.: G. et K. 2737-2740 (1965)	2n = 40
pop. 15	On a "legakker" near Vinkeveen (prov. of Utrecht), on peaty clay	
	coll. no.: G. et K. 1345-1349 (1964)	2n = 40
	coll. no.: G. et K. 4710-4716 (1966)	2n = 40
pop. 16	In the Eempolder (prov. of Utrecht), in various places	
a.	near Eembrugge, in a small marsh, on peaty clay	
	coll. no.: G. et K. 1150-1151 (1964)	2n = 40
	coll. no.: G. et K. 1796-1799 (1965)	2n = 40
b.	2 km E. of Bunschoten, at the border of a ditch, on peaty clay	
	coll. no.: G. et K. 1152-1155 (1964)	2n = 40
c.	near the mouth of the river Eem, at the border of a ditch, on peaty clay	
	coll. no.: G. et K. 1801-1824 (1964)	2n = 40
	coll. no.: G. et K. 1829-1830 (1964)	2n = 40
pop. 17	Near Broek op Langendijk (prov. of Noord-Holland), in two places, beside the canal Alkmaar-Kolhorn, on peaty and humus-rich clay	
	coll. no.: G. et K. 3359-3363 (1965)	2n = 40
	coll. no.: G. et K. 3365-3368 (1965)	2n = 40
	coll. no.: G. et K. 3364 (1965)	2n = 44

\*) legakker = longitudinal remnant of the original bog.

- pop. 18 At the border of a pool near Edam (prov. of N. Holland), on peaty clay  
coll. no.: G. et K. 1609 (1964) . . . . . 2n = 40
- pop. 19 At the border of a pool, 3 km S. of Hoorn (prov. of N. Holland), on clay-poor peat  
coll. no.: G. et K. 1144-1147; 1149 (1964) . . . . . 2n = 40
- pop. 20 In a dune-marsh S. of Callantsoog near the lake "het Zwanenwater", prov. of N. Holland, on non loamy fine sand, slightly to non calcareous  
coll. no.: G. et K. 1142-1143 (1964) . . . . . 2n = 40
- pop. 21 De Hool near Dalerveen (prov. of Drente), roadside; on loamy sand  
coll. no.: G. et K. 3940-3943 (1966) . . . . . 2n = 48
- pop. 22 Zuidbarge (prov. of Drente), roadside, on slightly loamy coarse sand  
coll. no.: G. et K. 3944, 3945 (1966). . . . . 2n = 48
- pop. 23 Roadside between Emmen and Sleen (prov. of Drente), on slightly loamy sandy soil  
coll. no.: G. et K. 4017; 4021-4024 (1966) . . . . . 2n = 48
- pop. 24 Roadside near Kampen (prov. of Overijssel), on young sea clay  
coll. no.: G. et K. 1252-1255 (1964) . . . . . 2n = 48
- pop. 25 In various places of the "Knardijk", between Harderwijk and Lelystad (prov. of Gelderland), on clay  
coll. no.: G. et K. 1163-1171 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 2201-2213 (1965) . . . . . 2n = 48  
coll. no.: G. et K. 4048-4051 (1966) . . . . . 2n = 48
- pop. 26 Roadside near Renkum (prov. of Gelderland), on river clay  
coll. no.: G. et K. 1210-1212 (1964) . . . . . 2n = 48
- pop. 27 Roadside S. of Arnhem (prov. of Gelderland), on river clay  
coll. no.: G. et K. 1213, 1214 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 1256, 1257 (1964) . . . . . 2n = 48
- pop. 28 Roadside near Hemmen (prov. of Gelderland), on river clay  
coll. no.: G. et K. 1215, 1216 (1964) . . . . . 2n = 48
- pop. 29 Foreland of the river Waal near Dodewaard (prov. of Gelderland), on river clay  
coll. no.: G. et K. 1217-1218 (1964) . . . . . 2n = 48
- pop. 30 Roadside near Ellecom (prov. of Gelderland), on river clay  
coll. no.: G. et K. 2047-2048 (1965) . . . . . 2n = 48  
coll. no.: G. et K. 2093-2096 (1965) . . . . . 2n = 48  
coll. no.: G. et K. 2098-2103 (1965) . . . . . 2n = 48
- pop. 31 In a meadow near Heelsum (prov. of Gelderland), on river clay  
coll. no.: G. et K. 4457-4459 (1966) . . . . . 2n = 48
- pop. 32 Near Bennekom (prov. of Gelderland), in a low meadow on loamy fine sand  
coll. no.: G. et K. 6039-6042 (1966) . . . . . 2n = 48
- pop. 33 Roadside in the Lobberdenwaard near Pannerden (prov. of Gelderland), on river clay  
coll. no.: G. et K. 4393-4397 (1966) . . . . . 2n = 48

- pop. 34 Roadside between Pannerden and Oud-Zevenaar (prov. of Gelderland), on river clay  
coll. no.: G. et K. 4378-4380 (1966) . . . . . 2n = 48
- pop. 35 Beside the Neerlangbroekse Wetering near Neerlangbroek (prov. of Utrecht), on heavy clay  
coll. no.: G. et K. 6035-6038 (1966) . . . . . 2n = 48
- pop. 36 At the border of the river Kromme Rijn near Bunnik (prov. of Utrecht), on river clay  
coll. no.: G. et K. 319 (1962) . . . . . 2n = 48
- pop. 37 Roadside between Jutphaas and IJsselstein (prov. of Utrecht), on river clay  
coll. no.: G. et K. 531 (1962) . . . . . 2n = 48
- pop. 38 Fort Hoofddijk near de Bilt (prov. of Utrecht), on river clay  
coll. no.: G. et K. 558 (1962) . . . . . 2n = 48  
coll. no.: G. et K. 1681 (1965) . . . . . 2n = 48
- pop. 39 In a small marsh beside the river Rhine near the Grebbeberg, Rhenen (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1194-1206 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 1768 (1965) . . . . . 2n = 48
- pop. 40 Roadside between Jutphaas and Houten (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1219-1221; 1224 (1964) . . . . . 2n = 48
- pop. 41 Beside the Amsterdam-Rijn kanaal near Schalkwijk (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1225-1228 (1964) . . . . . 2n = 48
- pop. 42 In a young *Populus*-wood near Tull en 't Waal (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1232, 1233, 1235, 1237-1239 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 3690-3700 (1966) . . . . . 2n = 48
- pop. 43 In a meadow in foreland of the river Lek near Lopik (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1241-1247 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 2121-2130 (1965) . . . . . 2n = 48
- pop. 44 Roadside near Utrecht, on river clay  
coll. no.: G. et K. 1304-1307; 1314 (1964) . . . . . 2n = 48
- pop. 45 At the margins of the wood of the estate "Oud Amelisweerd" between Utrecht and Bunnik (prov. of Utrecht), on river clay  
coll. no.: G. et K. 1308-1313 (1964) . . . . . 2n = 48
- pop. 46 Beside the river Hollandse IJssel near IJsselstein (prov. of Utrecht), on river clay (in the direct vicinity of population no. 1)  
coll. no.: G. et K. 1578 (1964) . . . . . 2n = 48
- pop. 47 In an osier-bed near Vreeswijk (prov. of Utrecht), on river clay  
coll. no.: G. et K. 3119-3123 (1966) . . . . . 2n = 48
- pop. 48 Roadside near the estate "Oostbroek" near de Bilt (prov. of Utrecht), on river clay  
coll. no.: G. et K. 3922-3923 (1966) . . . . . 2n = 48
- pop. 49 Roadside between Utrecht and Houten, (prov. of Utrecht), on river clay  
coll. no.: G. et K. 4154-4163 (1966) . . . . . 2n = 48

- pop. 50 3 km S. of Hoorn (prov. of N. Holland), on a clay-dike along the coast of the IJsselmeer, in the direct vicinity of population no. 19  
coll. no.: G. et K. 1143, 1148 (1964) . . . . . 2n = 48
- pop. 51 In various places on the Isle of Voorne (prov. of Z. Holland),  
a. Heveringen near Oost-Voorne, on sandy soil  
coll. no.: G. et K. 1273-1276 (1964) . . . . . 2n = 48  
b. Roadside near former airstrip, now a wet dune valley, near Oost-Voorne, on sandy soil  
coll. no.: G. et K. 1277-1289 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 4039-4042 (1966) . . . . . 2n = 48  
c. At the margin of a small *Populus*-wood, near Oost-Voorne, on sandy soil  
coll. no.: G. et K. 1899-1904 (1965) . . . . . 2n = 48  
d. At the border of the dune lake "Quakjeswater" on sandy soil  
coll. no.: G. et K. 1289, 1290 (1964) . . . . . 2n = 48  
coll. no.: G. et K. 1933-1937 (1965) . . . . . 2n = 48
- pop. 52 Near Beversluisplaat, Hollandse Biesbosch, on river clay. Plants regularly flooded at high tide  
coll. no.: G. et K. 1328 (1964) . . . . . 2n = 48
- pop. 53 Roadside N. of Goedereede, Isle of Goeree (prov. of Z. Holland), on sandy soil  
coll. no.: G. et K. 3142-3146 (1965) . . . . . 2n = 48
- pop. 54 a. Brabantse Biesbosch, polder "de Dood", on river clay. Plants regularly flooded at high tide  
coll. no.: G. et K. 1329-1333; 1336-1339 (1964) . . . . . 2n = 48  
b. Brabantse Biesbosch, in an osier-bed near Keizersveer, on river clay  
coll. no.: G. et K. 1334-1335 (1964) . . . . . 2n = 48
- pop. 55 Near Veldhoven (prov. of N. Brabant), on loamy fine sand  
coll. no.: G. et K. 2940-2941 (1965) . . . . . 2n = 48
- pop. 56 At the border of an old sea-creek near Woensdrecht (prov. of N. Brabant), on young sea clay. Only *Symphytum*-population in a brackish environment  
coll. no.: G. et K. 3147, 3148 . . . . . 2n = 48
- pop. 57 At the margins of a wood near Ulvenhout (prov. of N. Brabant), on loamy fine sand  
coll. no.: G. et K. 3917-3921 (1966) . . . . . 2n = 48
- pop. 58 Roadside near Heusden (prov. of N. Brabant), on river clay  
coll. no.: G. et K. 4201-4222 (1966) . . . . . 2n = 48
- pop. 59 At the margins of "de Geelders", a wood near Boxtel (prov. of N. Brabant), on loamy fine sand  
coll. no.: G. et K. 4233-4242 (1966) . . . . . 2n = 48  
coll. no.: G. et K. 4243 (1966) . . . . . 2n = 47
- pop. 60 At the margins of a small wood near Olland (prov. of N. Brabant), on very loamy fine sand  
coll. no.: G. et K. 4244-4248 (1966) . . . . . 2n = 48  
coll. no.: G. et K. 4249 (1966) . . . . . 2n = 47
- pop. 61 Near Helvoirt (prov. of N. Brabant), at the border of a small ditch, on slightly loamy coarse sand  
coll. no.: G. et K. 4264-4270 (1966) . . . . . 2n = 48

pop. 62	At the margin of a wood near Bunde (prov. of Limburg), on river clay	
	coll. no.: G. et K. 2582, 2583 (1965)	2n = 48
pop. 63	Roadside near Eys (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 2584 (1965)	2n = 48
	coll. no.: G. et K. 4088 (1966)	2n = 46
pop. 64	Roadside near Ryckholt (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 2604-2605 (1965)	2n = 48
pop. 65	Roadside near St. Geertruid (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 2612 (1965)	2n = 48
pop. 66	Roadside near Heerlerheide (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 4132, 4133 (1966)	2n = 48
pop. 67	Roadside between Heerlerheide and Hoensbroek (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 4134 (1966)	2n = 48
pop. 68	Roadside near Voerendaal (prov. of Limburg), on sandy loam	
	coll. no.: G. et K. 4136 (1966)	2n = 48
pop. 69	At the border of a small ditch near Ermelo (prov. of Gelderland), on slightly loamy sand soil	
	coll. no.: G. et K. 1158, 1159 (1964)	2n = 40
	coll. no.: G. et K. 1157 (1964)	2n = 42
	coll. no.: G. et K. 2195, 2196 (1965)	2n = ca. 42
	coll. no.: G. et K. 1160, 1161 (1964)	2n = 43
	coll. no.: G. et K. 2198 (1965)	2n = ca. 43
	coll. no.: G. et K. 2193, 2197, 2200 (1965)	2n = 44
	coll. no.: G. et K. 2194, 2201 (1965)	2n = 46
	coll. no.: G. et K. 2199 (1965)	2n = 47
	coll. no.: G. et K. 1162 (1964)	2n = 48
pop. 70	Beside a former riverbed near Oud-Zevenaar (prov. of Gelderland), on river clay	
	coll. no.: G. et K. 4412 (1966)	2n = 44
	coll. no.: G. et K. 4371, 4375 (1966)	2n = 46
	coll. no.: G. et K. 4377 (1966)	2n = 47
	coll. no.: G. et K. 4370, 4410 (1966)	2n = 48
pop. 71	On the Dolsberg between Gulpen and Wylré (prov. of Limburg), roadside, on loamy soil	
	coll. no.: G. et K. 2586 (1965)	2n = 43
	coll. no.: G. et K. 2397, 2585, 2586 (1965)	2n = 44
	coll. no.: G. et K. 2396 (1965)	2n = 45
	coll. no.: G. et K. 2588 (1965)	2n = 46
pop. 72	Roadside near Hoensbroek (prov. of Limburg), on loamy soil	
	coll. no.: G. et K. 4135 (1966)	2n = 44

In the table may be observed that five categories of populations can be distinguished with regard to the chromosome number:

- a. Two populations with 41 diploid plants, ( $2n=24$ ).
- b. Fifteen populations with 129 plants, ( $2n=40$ ).



- c. Forty-eight populations with 294 tetraploid plants, ( $2n=48$ ).
- d. Three mixed populations consisting of diploids and tetraploids.
- e. Four populations with plants with different chromosome numbers, varying from  $2n=40$  to  $2n=48$ .

It is clear that mixed populations are rare. Generally within the same population all individuals have the same chromosome number.

Only in diploid plants B-chromosomes have been found. These chromosomes are smaller than the chromosomes of the normal complement, have clear centromeres, and occur in various numbers (1-4). Especially in population no. 1, (IJsselstein) this phenomenon could be frequently observed, but plants with the exact diploid number  $2n=24$  are also met with.

Most tetraploid plants have exactly the chromosome number  $2n=48$ , but sometimes slight deviations occur. In the population no. 59 (Geelders near Boxtel) and in the population no. 60 (near Olland) one plant with the chromosome number  $2n=47$  was found, and in the population no. 63 (near Eys) one plant with the number  $2n=46$  was met with.

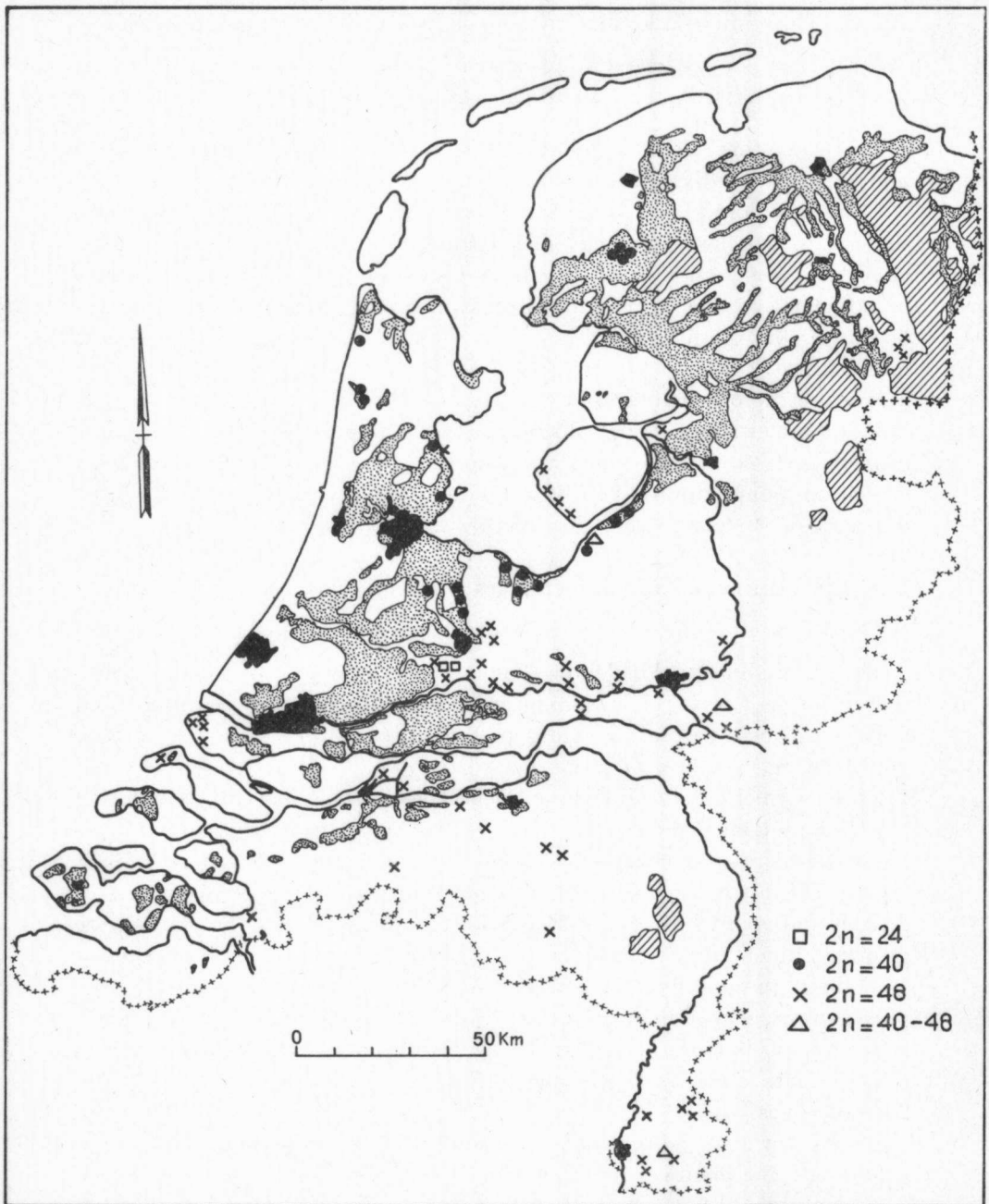
## II. The distribution of the cytotypes

The distribution of the various cytotypes is shown on the map.

This map and the table show that in general the  $2n=40$  type is represented on peat soils. These plants grow under very moist conditions and always in open vegetation. Only in two cases plants with the number  $2n=40$  were met with on very moist sandy soil (populations no. 12 and 20, from Ermelo and Callantsoog, respectively). The plants of population no. 16 (Eempolder) and of population no. 10 (between Elburg and Kampernieuwstad) grew on peaty clay, (under a clay layer of 40-80 cm a peat layer). The plants of these populations were restricted to the borders of ditches and pools. Both in the Eempolder and near Elburg the plants were growing under very moist conditions. Near Sneek (population no. 6-9), Vinkeveen (population no. 15), and Westbroek (population no. 13) the plants were very numerous on so called "legakkers", i.e. longitudinal remnants of the original bog. These facts suggest that the ecological requirements of the  $2n=40$  type differ from those of the  $2n=24$  and  $2n=48$  types.

Plants with the chromosome number  $2n=48$  were never found on peaty soil. The plants occur in dryer places than plants with the chromosome number  $2n=40$ , but they sometimes were found in very moist places. Tetraploids occur at roadsides, in or near woods (but never completely shaded), on dikes, in forelands of rivers, by pools and ditches (always on clayey and sandy soils), sometimes in places which are frequently flooded at high tide in the freshwater tidal delta "de Biesbosch".

Tetraploid plants grew sometimes intermingled with diploid plants,



The distribution of the investigated cytotypes of *Symphytum officinale* L. in the Netherlands. Finely stippled: low-moor peat; Cross-hatched: high-moor peat. (Edaphic data from: De Bodem van Nederland, p. 148, Wageningen, 1965).

(populations 3, 4, 5 near IJsselstein). Therefore it seems probable that diploid and tetraploid plants may have the same ecological requirements. An analysis of the vegetation in which the three cytotypes occur is planned.

### III. The interfertility of plants with different chromosome numbers

In view of the fact that in populations with diploids and tetraploids no intermediate chromosome numbers could be observed, it seems probable that, at least in the wild, no gene-exchange occurs. Several attempts to cross diploids and tetraploids failed, with one exception: From the cross plant no. 1336 (♂,  $2n=48$ ) X plant no. 1279 (♀,  $2n=24$ ) nine seeds could be obtained, only one of which proved germinable. This germinated several weeks after other, simultaneously sown seeds. The hybrid proved to be vigorous, but has not flowered so far. The chromosome number turned out to be  $2n=36$ . A description of the hybrid will be given later and the fertility will be tested experimentally.

In the populations 69, 70 and 71 the chromosome numbers range from 40–48. In some of these plants aneusomaty occurs. It is tempting to explain the origin of these numbers by crossing and subsequent backcrossing of the cytotypes  $2n=40$  and  $2n=48$ . Hybridization experiments revealed the fact that the cytotypes  $2n=40$  and  $2n=48$  are crossable in both directions. The hybrids are characterized by the number  $2n=44$ . The morphology of these hybrids is an object of further research. The fertility of these plants will also be tested, as well as the possibility of backcrossing with either parent.

### IV. Morphological data

#### a. *Height of the stems*

In the field the plants of the  $2n=40$  type tend to be lower than the diploid and tetraploid plants. This character, however, will be studied in plants which have been grown some years under the same conditions.

#### b. *Indument of the plants*

##### 1. Hairs of the upper surface of the leaves

The plants of the  $2n=40$  type have stems and leaves which are prickly, whereas the diploid and tetraploid plants have densely hispid, never scabrous, stems and leaves. In the  $2n=40$  type the hairs of the upper surface of the leaves are much shorter (max.  $3/4$  mm) than in the diploid and tetraploid plants (max.  $1\frac{1}{4}$  mm). The hairs of the  $2n=40$  type are deciduous in older leaves, whereas the hairs of diploids and tetraploids are generally persistent. The hairs have a tubercular base, which in the  $2n=40$  plants consists of three or more rings of cells, whereas in the diploid and tetraploid plants mostly one ring is present.

## 2. Hairs of the sepals

The hairs are not evenly distributed on the outer surface of the sepals. Three rows can be distinguished, two marginal and one dorso-medial. Between these rows irregularly scattered hairs are found. Three types of hairs can be distinguished:

- a. Unicellular, straight or slightly curved hairs of the same type as on the surface of the leaves. There are long ( $a_1$ ) and short ( $a_2$ ) hairs.
- b. Unicellular uncinat hairs, shorter than the straight ones.
- c. Glandular hairs with swollen apical cell.

The long hairs (type  $a_1$ ) are confined to the dorso-medial and marginal rows. In the  $2n=40$  type they are generally longer than in the  $2n=24$  or  $2n=48$  type. Between these long hairs (type  $a_1$ ) in the  $2n=40$  type one or two small straight (type  $a_2$ ) or uncinat hairs (type b) but never glandular hairs (type c) are found. More than two straight (type  $a_2$ ), uncinat hairs (type b) or—in many Dutch populations—glandular hairs (type c) are found in diploid and tetraploid plants.

In the  $2n=40$  type between the medio-dorsal and marginal rows only a few short straight (type  $a_2$ ) or uncinat hairs (type b) are met with, while in all investigated plants of the  $2n=24$  and  $2n=48$  type many straight (type  $a_2$ ) and uncinat hairs (type b) and often also glandular hairs (type c) occur.

The trichomes of the different cytotypes of different populations will be the object of further investigations.

### c. *The base of the stemleaves*

In the diploid and tetraploid plants the upper stemleaves are generally decurrent, whereas in the  $2n=40$  type they are not or only partially decurrent.

### d. *Number of inflorescences per plant*

In the field the  $2n=40$  type proved to have fewer inflorescences per plant than the individuals of the diploid and tetraploid type.

### e. *The colour of the corolla*

All diploid plants are white-flowered.

The  $2n=40$  type sometimes has also white flowers, but the majority have light, dark or red purple corollas. In the large population of the Eempolder (population no. 16), consisting of thousands of individuals, all flowers were light purple. White flowers were never observed. In hundreds of individuals of the population between Elburg and Kamper-nieuwstad (population no. 10) only one proved to be white-flowered. The only population in which some white-flowered plants were present is the population near Vinkeveen (population no. 15). Among 134 tetraploid

plants cultivated in the experimental garden 52 were pale purple, 43 white, 25 purple, and 14 red purple.

In the mixed population near Ermelo (population no. 69), with different chromosome numbers, the distribution of the colour of the corollas was as follows:

2n=42: light purple; 2n=43: purple; 2n=44: purple; 2n=46: white; 2n=47: white.

All (large) plants from the Dolsberg near Wylré (population no. 71) turned out to have the same colour: red, turning blue as the flowers aged.

#### *f. Other floral characters*

Different floral characters such as the size of the corolla, the length and the width of the corolla scales, the ratio of the length anther/filament, the shape of the calyx lobes, were subjected to biometric investigations. The results will be dealt with later. These studies pertain to some large diploid, tetraploid, and 2n=40 populations. Besides samples of flowers collected in nature also plants cultivated for some years under the same conditions as well as some hybrids and plants from abroad will be the object of such investigations.

### V. Flowering period

There is an indication that in the wild the flowering period of the 2n=40 type is shorter than that of the 2n=24 and 2n=48 type. In September generally all 2n=40 plants are past flowering. At the same time the other cytotypes are still in flower. These observations will be continued in the experimental garden.

### DISCUSSION

The above mentioned results demonstrate that the 2n=24 and the 2n=48 type are morphologically very similar and can easily be distinguished from the 2n=40 type. The plants of the 2n=40 type also prefer habitats in which diploid or tetraploid plants were never observed. It seems, therefore, that the 2n=40 plants have a different ecological preference. After an examination of some specimens, quoted by BUCKNALL (1913), e.g. *S. uliginosum* Kern., Herb. Gower, Caucasus, 1820, (K), *S. uliginosum* Kern., Vésztő, Hungary, Borbas, 1877, (K), we have come to the conclusion that the Dutch plants of the 2n=40 type closely resemble these specimens. The specimens examined by Bucknall are somewhat more slender, whereas the mediodorsal row of hairs of the outer surface of the sepals is less densely haired. Moreover, the plants of the 2n=40 type largely match the original description of *S. uliginosum* Kern. Comparison with the type material, however, is necessary before further conclusions can be drawn.

An interesting problem is formed by the distribution area of *S. uliginosum*. According to BUCKNALL (l.c.) this species occurs in Hungary and in

the South of Russia. Some forms which resemble *S. uliginosum* to a certain extent are found in Austria, France (Alsace) and in the West of Switzerland (HEGI, 1927). Probably these forms are not to be regarded as indigenous but as naturalized (HEGI, l.c.). ROTHMALER (1963) is of the opinion that *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. occurs in the Rhine province of Western Germany. Therefore, it is not impossible that the range of *S. uliginosum* extends to Western Europe. Herbarium studies are necessary to solve this problem.

The morphological and cytological characteristics as well as the ecological preferences of the  $2n=40$  type seem to support the treatment of this cytotype as a separate species. On the other hand, the crossability of the  $2n=40$  and the  $2n=48$  type indicate that these types are better regarded as conspecific. A study of the fertility of the hybrid, including its morphological characters, is necessary before it will be possible to arrive at more definite conclusions.

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