Accessory Chromosomes in Norwegian Populations of *Paris quadrifolia* L.

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Accessory chromosomes (ref. Håkansson 1945, Müntzing 1945, Müntzing 1974) are now known from numerous plant species. In 1964 Battaglia published a list of 160 species of higher plants with occurrence of such chromosomes, and only a few years later the number had increased to about 590 species belonging to 219 genera (Jones 1975).

Accessory chromosomes are not necessary for the life of the individual and may be present in only certain tissues. They are further subinert, having weak—sometimes distinct genetic effects. These chromosomes may also reduce the fertility (Battaglia 1964, Rees 1974) and sometimes increase the frequency and distribution of chiasmata (Vosa and Barlow 1972, Müntzing 1974, Rees 1974, —see also Dyer 1979).

It is frequently found that they are heterochromatic and are often of considerably smaller size than the members of the regular chromosome complement.

The origin and formation of accessory chromosomes are largely unknown. Recently, new theories have been proposed involving a possible origin of accessory chromosomes by breaks in meta- and acrocentric chromosomes together with centric fusion (Jones 1978).

The complex problems involved with accessory chromosome formation and behaviour are naturally most favourably analyzed in plant species with very large chromosomes. In the tribe Paridae of the family Liliaceae the genera *Trillium* and *Paris* have almost the largest chromosomes known in the plant kingdom (Huskins and Wilson 1938, Geitler 1937, 1938). Accessory chromosomes have previously been reported from *P. polyphylla* and *P. tetraphylla*. We report such chromosomes in a third species *P. quadrifolia* L. This is widely distributed all over the country from the southernmost parts of Norway to the arctic northern areas, being rare on the western coast, but may occur in the central mountain area up to 1200 m above sea level.

Materials and methods

Populations from 26 different localities (Table 1, Fig. 1) were analyzed and grown in green-houses at the Botanical Laboratory, University of Oslo. More than 10 different specimens were studied from each population. Totally about 800 specimens were examined.

Root-tips were pre-treated in 0, 2% colchicine in distilled water for about 3 hours at 20°C, then fixed in acetic-ethanol for about 48 hours. The specimens were then preserved in 70% ethanol until examination.

Table 1. Survey of material examined. Chromosome accounts are added.
Paris quadrifolia L.

Population no.	Locality	Habitat	Chromosome no.	No. of examined specimens
P1	Kirkenes, Finnmark	Birch forest, course of brook	2n=20	12
P2	Talvik, Alta, Finnmark	Pine-alder forest, moist soil	2n=20	12
P3	Tromsø, Troms	Birch forest among small ferns	2n=20+10 acc.	200
P4	Søreide, Gloppen, Sogn & Fjordande	Deciduous forest, moist, sunny	2n=20	13
P5	Voss, Hordaland	Spruce forest, shady	2n=20	16
P6	Voss, Hordaland	Deciduous forest, moist soil, shady	2n=20	18
P7	Bergen, Hordaland	Deciduous forest, moist soil, shady	2n=20	31
P8	Fana, Hordaland	Deciduous forest, moist soil, shady	2n=20	12
P9	Stord, Hordaland	Deciduous forest, moist soil, shady	2n=20	30
P10	Røldal, Hordaland	Among tall herbs, moist soil, shady, stony place 700 m	2n=20	11
P11	Farsund, Vest-Agder	Deciduous forest, moist soil	2n=20	11
P12	Arendal, Aust-Agder	Wet Picea forest	2n=20	14
P13	Lillesand, Aust-Agder	Meadow on limestone	2n=20	14
P14	Gjerstad, Aust-Agder	Picea forest with herbs (Melico-Picetum)	2n=20	12
P15	Nome, Telemark	Mixed forest, dry, sunny place	2n=20	20
P16	Nome, Telemark	Picea forest with herbs (Melico-Picetum)	2n=20	30
P17	Siljan, Telemark	Picea forest with herbs (Melico-Picetum), shady place, moist soil	2n=20+1 acc.	100
P18	Skien, Telemark	Picea forest with herbs (Melico-Picetum), shay place, moist soil	2n=20	25
P19	Skien, Telemark	Picea forest with herbs (Melico-Picetum) shady place, moist soil	2n=20+2 acc.	100
P20	Rygge, Ostfold	Deciduous forest, shady, moist soil	2n=20	25
P21-23	Oslo, different localities	Deciduous forest, shady	2n=20	20-20-15
P24	Blankvann, Oslo	Marsh, soil containing lime, moist and exposed to sun	2n=20	15
P25	Svartor, Oslo	On limestone, dry, exposed to sun	2n=20	15
P26	Hønefoss, Buskerud	Deciduous forest, shady place	2n=20	14

The material was squashed in 45% acetic acid and stained in 2% orcein. The preparations were then mounted in Euparal.

Meiotic cells were simply dissected out by aid of two needles, fixed in 45% acetic acid on the slide and stained by adding a drop of aceto-orcein. The preparations were mounted in Euparal.

Microphotos were made by a Zeiss Photomicroscope II, using an oil immersion planapochromatic lens 63/1, 40 and Kodak Plus X (22° DIN) film.

Observations and discussion

All populations of *Paris quadrifolia* examined were on the "tetraploid" level with the somatic chromosome number 20 (ref. Table 1).

Fig. 1. Accessory chromosomes in Pop. 19 (A, B) and Pop. 17 (C) of *Paris quadrifolia* L. (A, B) show second meiotic division, (C) shows mitosis and interphase in root-tip cells.

The chromosomes may be arranged in five groups of four, reflecting an allotetraploid origin. Especially in the short arms the four members of each type of chromosomes differ in the manner expected for an allotetraploid. In principle all populations contained 4 large, metacentrics (A-group)+4 large submetacentrics (B-group)+4 medium submetacentrics (C-group)+4 medium acrocentrics (D-group)+4 medium-subtelocentrics (E-group)+accessories (f):

$$4L_m + 4L_{sm} + 4M_{sm} + 4M_a + 4M_{st} + (acc.)$$

(A) (B) (C) (D) (E) (f)

The most distinct differences inside the groups are in the short arms of the E-group where two chromosomes usually lack the NOR-region.

There are characteristic variations in karyotypes between different populations, due to presence of unequal long and/or short arms inside the groups. These are considered mostly to be due to gross structural rearrangements.

Fig. 2. Accessory chromosomes in pollen mother cells of Pop. 3 of *Paris quadrifolia* L. Note variations in numbers and size. Arrows point at probable constrictions. The numbers varied mostly between 3 (C, D, H) and 9 (E).

Fig. 3. Map of Norway showing the distribution of the examined populations.

The average length of chromosome arms in different groups were calculated by Darlington (1941) as:

(A) 9.5+9 (for 4 chr.)	(B) 7.5+5 (4 chr.)	(C) 5.5+3.5 (4 chr.)	(D) 9+2.5 (4 chr.)
(E) 9+0.5 (4 chr.)			

These values in μm appears to represent the usual situation in the Norwegian *Paris* karyotypes. However, about 20% of the Norwegian material shows karyotype deviations. Only one extreme example will be mentioned:

Population (12):

(A) 0.2+7 (3 chr.)	and	9.5+9 (one chr.)	(B) 7.5+5 (4 chr.)	(C) 5.5+3.5 (4 chr.)
(D) 0 (missing)	and	9+2.5 (3 chr.)	(E) 9+0.5 (4 chr.)	

Table 2. Number of accessory chromosomes in somatic and meiotic cells of *Paris quadrifolia* L.

Population no.	Number of plants containing acc. chrom.	Number of examined plants	Number of acc. chrom.	
			in root-tip cells	in PMC
3	3	200	0	10
17	1	100	1	0
19	1	100	0	2

Table 3. No. of accessory chromosomes in PMC's from Pop. No. 3.

Plant no.	No. of cells containing acc. chrom.	Highest no. of acc. chrom. in one cell	Most common no. of acc. chrom. per cell
1	18	9	1
2	12	10	1
3	38	10	2

The accessory chromosomes (f-group) were present in three populations. They were found in either or both meiotic and somatic cells. (Pop. 3, Tromsø, County of Troms, Pop. 17, Siljan, County of Telemark and Pop. 19, Fjelldalen, County of Telemark, ref. Table 1.)

In population No. 19 the number varied between two and zero in the PMC's (Fig. 1, A-B). In population No. 17, however, (Fig. 1C) a single accessory chromosome was found in some root-tip cells of one individual.

The accessory chromosomes of *Paris quadrifolia* differ markedly from the chromosomes belonging to the normal complement. Besides being much smaller (size 1-2 μm) they are of globular shape and are usually heterochromatic. Their shape varies slightly between cells and different tissues. In PMC's in meiotic prophase (Fig. 2, A-D) condensed chromatin bodies are present *outside* the leptotene euchromosome group. These bodies, regarded as accessories, show sometimes by close microscopy a distinct differentiation in chromatids (ref. lower group, Fig. 2, D-E-I) clearly demonstrating their chromosomal nature. They are persistent

in condensed state in later meiotic stages (Fig. 2, I-diplotene) and were not observed to split during meiotic divisions. Also in later meiotic stages they remained highly condensed (Fig. 1, A–B).

A similar behaviour was found in the root-tip cells. The accessory material remains condensed outside the interphase nucleus (Fig. 1C).

Numerical variations in the number of accessory chromosomes are present between different specimens in a single population (Table 2), as well as between different cells.

It is evident that accessory chromosomes are rarely occurring in *Paris quadrifolia*. They were found in only 1–3% of the analyzed specimens. We suggest, however, that it may be possible to relate very closely the origin of such accessories to the large structural changes observed in this species. This necessitates extensive cytological studies, including banding techniques. The extreme chromosome size in *Paris quadrifolia* thus represents an unusual potential for a close examination of the origin of accessory chromosomes. Further studies are thus in progress.

Summary

We report presence of accessory chromosomes in some populations of *Paris quadrifolia* L. In one population up to ten such chromosomes occurred in the PMC's. These chromosomes were also found occasionally in root tip cells, being condensed outside the nuclear membrane. The number of accessories varied both between meiotic and somatic cells. *Paris quadrifolia* L. is a species well-known for its particular large chromosomes. The extensive structural differences of karyotypes between different populations in Norway might give interesting clues to the origin of accessories.

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