THE CYTOLOGY OF *CALTHA PALUSTRIS* : CYTOGENETIC RELATIONSHIPS

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1. INTRODUCTION

SEVERAL workers have studied the cytology of *Caltha palustris* L. sensu lato. The results are summarised in Kootin-Sanwu and Woodell (1970). No hybridisation between the chromosome races had been carried out until 1963, when we initiated a hybridisation programme. Recently Wcislo (1968) has made crosses between two chromosome races in Europe. In this paper we shall discuss our results and their implications.

2. MATERIALS AND METHODS

Parent plants were obtained from the field, potted and maintained in a greenhouse. The pots were placed in shallow water, since this species is semi-aquatic. The crosses involved the $2n = 32$, $2n = 56$ and $2n = 64$ races, and some F_1 progeny. For meiotic analysis buds were fixed in Carnoy's fixative and anthers stained in acetocarmine. Somatic counts were made from Colchicine-treated root-tips fixed in acetic-alcohol and stained by the Feulgen technique after hydrolysing in N.HCl for about 6 minutes at 60°C .

3. PRELIMINARY EXPERIMENTS

(a) Bagging and emasculation experiments showed that all the chromosome races are self-incompatible and it is unlikely that autonomous apomixis occurs. Wcislo (1968) found that the $2n = 32$ and $2n = 56$ races are self-incompatible.

(b) Mature seeds were collected from a $2n = 56$ plant in the wild and germinated in garden soil. The chromosome numbers of the seedlings were determined (table 1). A very high proportion (68.4 per cent.) of the progeny had chromosome numbers different from that of the parent, and 63.1 per cent. had supernumerary chromosomes (Kootin-Sanwu and Woodell, 1969). It is known that sexual and multivalent-forming polyploids such as *Dactylis glomerata* and *Anthoxanthum odoratum* occasionally give rise to aneuploid offspring. Aneuploid offspring are also common in some apomictic species (Jones, 1957).

(c) Percentage pollen fertilities were determined by staining fresh pollen grains in acetocarmine; those taking up the stain were classed as fertile. Colourless, pale, misshapen or shrunken grains were classed as infertile. Fertility is high irrespective of polyploids: two $2n = 64$ plants had a mean

fertility of 89.0 per cent., 98 $2n = 56$ plants from widespread localities were 94.6 per cent. fertile, and 17 European $2n = 32$ plants had a mean pollen fertility of 93.3 per cent.

(d) Since we believe the basic number of the species is 8, and the 56-chromosome race to be an odd-numbered polyploid, psuedogamy was initially suspected, especially since individuals are highly pollen fertile. Earlier reports (Mottier, 1895; Thomas, 1900; Grafl, 1941; Kapil and Jalan, 1962) indicate that the development of the female gametophyte of *Caltha palustris* L. follows a perfectly normal sexual pattern. The chromosome number of the material used was determined in none of these investigations, however.

TABLE 1

Chromosome numbers of seedlings from $2n = 56$ seed parent. (Seeds were collected from the wild.)

Number of seedlings	Somatic chromosome number
1	55
2	56
3	56+1B
1	56+3B
4	57
2	57+2B
4	57+3B

Flower buds from $2n = 56$ parent plants were collected in spring and fixed in F.A.A. Customary dehydration and embedding procedures were followed. Sections were cut at 10μ and stained with safranin and fast green.

The development of the female gametophyte follows the "*polygonum*" type and is normal. Some of the stages in development are shown in fig. 1.

4. RESULTS OF ARTIFICIAL CROSSES

(i) 56×64 -chromosome races

Thirteen crosses with 56-chromosome female parents, and one with a 64-chromosome female parent were successful. Hybrids from five of the crosses were analysed cytologically (table 2).

The chromosome numbers of these hybrids ranged from $2n = 56$ to $2n = 63$ (fig. 2). Such a range in two levels of ploidy has been reported elsewhere. In some cases, such as the *Luzula campestris-multiflora* complex (Nordenskiöld, 1956), intermediate chromosome numbers are found in the wild whenever two high polyploid races grow together. Such natural hybrids between two cytotypes of *Caltha palustris* L. have never been found. Usually different cytotypes are not found together, but Wcislo (1967) found mixed populations of the 32- and 56-chromosome races. We have found plants with $2n = 57, 58, 60$ and $60+3B$ growing in different populations together with the 56-chromosome race. Plants with such numbers are probably aneuploids since only one small population of the 64-chromosome race has been found in Britain, and since individuals with $2n = 52, 54$ and 55 have also been found in Britain.

Meiosis was very irregular in the hybrids examined (table 3). The frequency of univalents was often especially high. Apart from bivalents,

FIG. 1.—Stages in the development of the female gametophyte of *Caltha palustris* L. ($2n = 56$).

1. A megaspore mother-cell (*a*) in the archesporium.
2. Bisporic stage. The two cells (*b*) resulted from the division of the megaspore mother-cell. A vacuole (*c*) is developing.
3. The functional megaspore (*d*) undergoing division.
- 4 to 7. Further divisions of cells derived from the megaspore and the growth of the embryo sac (cf. *e* and *g*).
7. A mature embryo sac, with a prominent central nucleus: (*i*) egg cell, (*h*) with two synergids at the micropylar end, (*f*) and the antipodals, (*j*) at the opposite end of the embryo sac.

N.B.: Drawings are from vertical sections of young ovules. Magnification is $\times c.160$.

trivalents and quadrivalents, there were occasional higher associations (fig. 3). Stickiness occurred in some hybrid pollen mother-cells.

TABLE 2

Analysis of chromosome numbers at metaphase-I in F_1 hybrids from $2n = 64 \times 2n = 56$ and reciprocal crosses

Type of cross and somatic chromosome numbers of parents	Chromosome numbers of F_1 progeny								Number of plants
	56	57	58	59	60	61	62	63	
56×64	3	1	1	—	3	3	2	—	13
56×64	—	—	1	—	5	1	3	—	10
56×64	—	1	1	1	2	3	—	—	8
56×64	4	1	2	—	7	3	7	1	25
64×56	2	1	—	2	6	5	—	1	17
Totals	9	4	5	3	23	15	12	2	73

Anaphase was often very anomalous. In one $2n = 60$ hybrid, chromosome fragmentation was observed at anaphase-II and many pollen mother-cells were multinucleate after meiosis, with micronuclei in some cases. This was probably a consequence of the behaviour of the many lagging chromosomes seen at meiotic anaphase in some pollen mother-cells.

FIG. 2.—Root-tip meiosis of a $2n = 61$ F_1 hybrid from a $2n = 56 \times 2n = 64$ cross in *Caltha palustris* L. (Colchicine treated).

Pollen fertility of the hybrids ranged between 45 and 80 per cent. (table 4); much lower than that of the parents. Two $2n = 61$ individuals were aberrant, with tiny petaloid leaves. These flowered unusually early,

even before the first few leaves had opened, and they were completely male sterile. All the other hybrids were vegetatively vigorous.

(ii) 32×56 -chromosome races

A total of 35 crosses was made. Of 16 using a tetraploid ($2n = 32$) as female parent, only one failed; in 19 reciprocal crosses, only 10 were successful. In successful crosses, many seeds were shrivelled or misshapen. Chromosome numbers of hybrid individuals from seven crosses ranged from $2n = 41$ to $2n = 45$ (table 5). About 73 per cent. of the hybrid individuals had the number $2n = 44$ (fig. 4).

TABLE 3
Chromosome pairing in F_1 hybrids from $2n = 56 \times 2n = 64$ crosses

Somatic chromosome numbers of hybrids ($2n$)	Number of P.M.C.s	Mean chromosome pairing at metaphase-I (ranges in brackets)							
		I	II	III	IV	V	VI	VII	VIII
56	15	2.8 (0.12)	20.9 (14.19)	0.7 (0.9)	1.9 (0.5)	—	—	0.1 (0.1)	0.1 (0.1)
57	3	3.3 (1.8)	22.3 (21.23)	0.3 (0.1)	1.0 (0.3)	—	0.7 (0.1)	—	—
58	5	3.4 (1.6)	12.8 (8.18)	0.2 (0.1)	6.6 (3.9)	0.4 (0.1)	—	—	—
59	7	4.9 (3.7)	14.4 (10.16)	1.1 (0.2)	4.6 (4.5)	0.14 (0.1)	0.14 (0.1)	0.3 (0.1)	—
60	12	4.3 (1.7)	17.8 (15.22)	1.6 (0.4)	3.8 (2.6)	—	—	—	—
62	14	11.7 (0.46)	16.6 (8.23)	0.7 (0.3)	3.6 (0.8)	—	0.07 (0.1)	—	—

Meiosis was examined in two individuals, with $2n = 42$ and $2n = 44$. Pairing was very variable; univalents were common in most pollen mother-cells (table 6). However, anaphase appeared remarkably normal. Although the hybrids were very vigorous, they were all completely sterile.

Our results contrast strikingly with those of Wcislo (1964, 1968). She crossed only the 32- and 56-chromosome races. Every hybrid she examined cytologically had $2n = 44$. She analysed 49 pollen mother-cells and found no association higher than a bivalent. The perfect regularity in chromosome number of the hybrid and the absence of any associations other than univalents and bivalents is puzzling, in the light of our results. Wcislo (1967) and Smit (1967) examined plants of both these races. Wcislo's collections were from Poland, Bulgaria and Czechoslovakia. Of 1200 plants, only eight aneuploids were found, and no B chromosomes. Smit counted several hundred plants from Holland, Spain, Portugal, Germany, France, Austria and Finland. She found no aneuploids and no B chromosomes. This uniformity is very surprising in view of our results (nearly 4 per cent. aneuploids, over 20 per cent. plants with B chromosomes). Further, Reese (1954) from German material, and Maugini (1953) and Leoncini (1951) using plants from Austria and Italy, found many aneuploids and B chromosomes.

(iii) 32×64 -chromosome races

In 10 crosses using the tetraploid ($2n = 32$) as female parent, only one succeeded; three seeds were set and failed to germinate. Two reciprocal crosses failed completely.

FIG. 3.—*Caltha palustris* L. Meiosis in a pollen mother-cell of a $2n = 59$ F_1 hybrid from a $2n = 56 \times 2n = 64$ cross; metaphase-I with 1 (VII) + 5(IV) + 1(III) + 13(II) + 3(I)

TABLE 4

Percentage pollen fertility (by acetocarmine test) of F_1 hybrids from crosses involving the heptaploid and octoploid races of *Caltha palustris* L.

Number of hybrids	Chromosome number ($2n$)	Mean percentage pollen fertility
7	56	57.1
5	57	71.2
5	58	79.3
3	59	46.2
6	60	62.7
9	61	48.2
5	62	74.3
2	63	51.0

TABLE 5

Chromosome numbers of F₁ hybrids of $2n = 56 \times 2n = 32$ and reciprocal crosses

	Chromosome numbers of F ₁ hybrids					Number of plants
	41	42	43	44	45	
32 × 56	—	3	5	10	1	19
32 × 56	1	—	—	7	2	10
32 × 56	—	1	—	12	1	14
32 × 56	—	—	3	15	—	18
56 × 32	—	1	6	10	—	17
56 × 32	—	—	—	9	1	10
56 × 32	—	1	1	12	1	15
Total	1	6	15	75	6	103
Percentage of Total	0.97	5.82	14.56	72.81	5.82	—

FIG. 4.—Root-tip mitosis of an F₁ hybrid *Caltha palustris* L. $2n = 44$ from a $2n = 32 \times 2n = 56$ cross (colchicine-treated root tip).

TABLE 6

*Chromosome pairing at metaphase-I in $2n = 42$ and $2n = 44$ hybrids.
(Ranges are given in brackets)*

F ₁ hybrid and chromosome no.	Mean chromosome pairing at metaphase-I							Number of P.M.C.s
	I	II	III	IV	V	VI	VII	
$2n = 42$	7.3 (3-11)	9.9 (9-15)	1.4 (0-3)	2.5 (0-5)	0.1 (0-1)	— —	— —	8
$2n = 44$	6.3 (2-13)	9.7 (1-15)	2.2 (1-4)	2.6 (1-7)	— —	0.1 (0-1)	0.1 (0-1)	9

(iv) *Crosses within the 32-, 64- and 56-chromosome races*(a) *The 64-chromosome race*

Crosses failed between the only two 64-chromosome individuals found in this investigation. The plants came from the same population and possibly were from the same clone.

(b) *The 32-chromosome race*

Five out of seven pollinations within this race were successful. F_1 hybrids from one of these crosses proved to be uniformly tetraploid.

(c) *The 56-chromosome race*

Three types of crosses were made to test fertility and seed set within this race. The first was a series involving plants from the same populations; 14 out of 15 were successful. Seed set was heavy. Secondly, 13 interpopulation crosses were made. Inter- as well as intrapopulation hybrids were vigorous. Some showed hybrid vigour, with plant parts larger than those of the parents. F_1 hybrids from crosses between lowland and mountain forms were robust and showed no intermediate features. This presumably is related to the fact that the dwarf montane forms are ecologically dwarfed and increase in size and number of parts when grown in cultivation (Woodell and Kootin-Sanwu, 1971). Pollen fertility was over 90 per cent. in all the hybrids tested; in this we agree with Wcislo (1968).

One of the interpopulation crosses had a parent of the node-rooting type (var. *radicans* (Forst.) Beck) from Hopper's Wood, Co. Durham. Of 20 hybrids, six were node-rooting. This indicates that some of such hybrids cannot be detected in the wild.

The third series of crosses within this race involved eight pairs of sister F_1 interpopulation hybrids. Ten out of 16 crosses were successful. The F_2 plants were vigorous and free-flowering. Most had pollen fertilities over 90 per cent. In one series involving a cross between a lowland and a dwarf mountain strain, the pollen fertility of the F_2 individuals ranged from 45 to 90 per cent. with a mean for 20 plants of 63 per cent. This is much lower than that for the corresponding F_1 hybrids. It is in contrast with the restoration of fertility from a low level in the F_1 hybrid to higher levels in later generations (Heiser, 1951; Gajewski, 1957; Moore, 1959). In hybrids between *Festuca pratensis* and *Lolium perenne*, Peto (1933) found a lowered pollen fertility restored in the back-crosses (though some back-cross individuals had very low percentage fertility) and in *Primula* (Woodell, 1965) a similar situation occurred.

Several F_2 individuals from this series, with a drop in pollen fertility, were examined at meiosis. They had many meiotic abnormalities such as irregular anaphases and lagging chromosomes, spindle abnormalities and multinucleate telophases. These disturbances are probably the result of genotypic unbalance following recombination between differently balanced genotypes. Wcislo (1968) reported no such disturbances.

(v) *Fertility of the hybrids*

As we stated earlier, the hybrids from the $2n = 32 \times 2n = 56$ cross were all completely pollen sterile as Wcislo (1968) also found. She did succeed

in germinating three seeds from a number of interhybrid crosses. Two survived and had $2n = 44$ but they were stunted.

We tested the fertility of the $2n = 62$ and $2n = 60$ hybrids from the 56×64 chromosome race cross. An F_1 individual, $2n = 62$, was back-crossed to a $2n = 56$ parent. Five seeds were set, all non-viable. Four reciprocal crosses were unsuccessful. Four crosses of the type $2n = 60 \times 2n = 60$ were made. These were successful but average seed set was low.

(vi) *Seed set in crosses within and between the chromosome races*

Table 7 summarises the results of the crosses. In the wild the mean number of seeds per follicle ranges between five and twelve, so our crosses between parents of like ploidy produce normal seed set. In crosses between

TABLE 7

Success and seed-set in crosses within and between chromosome races of Caltha palustris L.

	Number of flowers pollinated	Number of successful crosses	Number of follicles in successful crosses	Total number of seeds	Number of good seeds	Average number of good seeds per follicle
32×32	7	5	47	373	373	7.9
32×56	16	15	212	509	172	0.8
56×32	19	10	119	466	82	0.7
32×64	10	1	8	3	3	0.4
64×32	2	0	0	0	0	0
$56 \times 56a^*$	15	13	121	1193	1185	9.8
$56 \times 56b^*$	13	12	91	661	652	7.2
$56 \times 56c^*$	16	10	80	544	526	6.6
56×64	13	13	48	237	221	4.6
64×56	1	1	6	38	31	5.1
64×64	2	0	0	0	0	0
$56 \times 62F_1$	1	1	12	5	0	0
$62F_1 \times 56$	4	0	0	0	0	0
$60F_1 \times 60F_1$	4	4	37	106	105	2.8

$a^* =$ Intrapopulation; $b^* =$ Interpopulation; $c^* =$ Interpopulation F_1 plants.

different chromosome races, those between 56 and 64 produced a reduced, but still quite good seed set, whichever race was used as female parent. However, in the cross between 32- and 56-chromosome races the seed set was very low. This result is comparable to many crosses between races or species of different ploidy; further the cross between tetraploid and octoploid was even less successful. In both $2n = 56 \times 2n = 32$, and $2n = 64 \times 2n = 32$ crosses, there was slightly higher success when the plant with lower ploidy was used as female. In their review of diploid-autotetraploid crosses, Woodell and Valentine (1961) noted that there was a tendency for crosses to be more successful when the tetraploid was used as female parent. Not enough data are available for *Caltha palustris* to establish whether it fits with this trend.

5. DISCUSSION

(i) *The status of the chromosome races*

The 56-chromosome race is almost the only one in Britain. We have found one population with $2n = 64$, and we have been unable to confirm Panigrahi's (1953) numbers of 72 and 80. In North America $2n = 32$ is the

common race; $2n = 56$ and about 64 have been reported. In Europe both the $2n = 32$ and $2n = 56$ races are widespread. Apart from these, plants with $2n = 16, 48, 60$ and 72 have been found, as well as two with $2n = 28$, and many aneuploids.

It is clear that the only two races that are successful are those with $2n = 32$ and $2n = 56$. The others are rare.

The tetraploid forms only bivalents during meiosis, and possesses a pair of satellited chromosomes in its mitotic complement. It can be argued from these observations and from the discovery of diploid forms (Leoncini, 1951; Packer, 1964) as well as the rarity of true autopolyploids in nature (Stebbins, 1960) that it is an allotetraploid. The parentage of the diploid hybrid from which it was formed is of course unknown. The rarity of the diploid race and the exclusive bivalent formation in the tetraploid suggest that the latter has evolved from a sterile or nearly sterile hybrid between two diploid species.

The origin of the 56-chromosome race has been discussed by several authors (Leoncini, 1951; Reese, 1954; Smit, 1967; Wcislo, 1968). There are two clear alternatives; either it is a heptaploid, assuming the basic number of the Genus to be 8, or it is an octoploid, in which case *Caltha* would have two basic numbers, 7 and 8.

There is no direct cytological evidence on this problem. We have attempted karyotype analyses; they reveal that there is no morphological or distributional pattern in the chromosomes which would help. Genomic constitutions of polyploids can often be determined by means of crosses with putative parents. This cannot be done in the case of *Caltha*. Therefore we can only weigh evidence for each alternative, and most of the evidence is circumstantial.

There are precedents for the occurrence of two basic chromosome numbers in a genus of flowering plants. In the Ranunculaceae several genera have basic numbers of 7 and 8, including *Ranunculus* (Gregory, 1941). They are all larger genera than *Caltha*. Within *Caltha*, all the species that have been counted other than *C. palustris* have a basic number of 8. The case for a basic number of 7 rests on two chromosome counts of $2n = 28$ for *C. palustris*. One of these is an unpublished count by Nygren, referred to by Löve and Löve (1948) and the other was a single count made by Sorsa (1962) on Finnish material. No study of meiosis has been made for this cytotype.

The $2n = 56$ chromosome race has somewhat irregular meiosis (Kootin-Sanwu and Woodell, 1970). The number of bivalents can be as low as 8, and quadrivalents as high as 10, with small numbers of uni-, tri-, penta- and hexavalents (table 8). In addition a high number of aneuploid seedlings is produced, most of which are eliminated in the wild (table 1).

Wcislo (1968) was able to obtain some pollen-fertile $2n = 44$ hybrids from the cross between the 32- and 56-chromosome races. Experimental back-crosses involving these hybrids included plants with $2n = 38$ (fusion of two normal gametes, $n = 16$ and $n = 22$); $2n = 50$ (fusion of two normal gametes, $n = 22$ and $n = 28$); and $2n = 72$ (fusion of a normal gamete of the non-hybrid parent, $n = 28$, with an unreduced hybrid gamete, $n = 44$). This indicates that although this hybrid is very unstable and highly infertile, there exists the possibility of formation of new chromosome races by hybridisation and the production of unreduced gametes. The presence of occasional hexaploids in the wild may be the result of fusion of reduced and unreduced

tetraploid gametes. The fusion of an unreduced tetraploid gamete with a normal hexaploid gamete could produce a heptaploid ($32 + 24 = 56$).

The irregular meiotic behaviour, the production of a large number of aneuploids, the fact that 8 is the basic number of the genus, and the apparent frequency of unreduced gametes under experimental conditions, lead us to the view that the $2n = 56$ race is a heptaploid. Finally, the occurrence of two basic numbers *within* a species is unusual.

Although univalents, trivalents and higher associations are formed at meiosis, the heptaploid is highly fertile, but the number of bivalents is relatively high, and the fertility is not surprising. Nor is this the only fertile and successful uneven polyploid. Pentaploid ($2n = 35$) and particularly heptaploid ($2n = 49$) *Holcus mollis* are fertile (Jones, 1958). Successful crosses involving the pentaploid *Agrostis stolonifera* ($2n = 35$) with two

TABLE 8

*Chromosome pairing at meiosis in the $2n = 56$ race of *Caltha palustris* L.*

Locality	Number of P.M.C.s	Mean metaphase pairing per pollen mother cell					
		I	II	III	IV	V	VI
Cothill, Berks	15	0.53 (0.2)	17.20 (12-24)	0.73 (0.4)	4.33 (0.10)	0.07 (0.1)	0.20 (0.2)
Islip, Oxon	15	0.93 (0.3)	17.93 (12-24)	0.80 (0.4)	4.20 (2.6)	—	—
Parks, Oxford	20	0.55 (0.2)	17.85 (12-22)	0.25 (0.2)	4.75 (3.7)	—	—
St Neots, Camb.	20	0.65 (0.2)	18.15 (8-24)	0.55 (0.3)	4.35 (2.10)	—	—
Memus, Forfar	14	0.21 (0.2)	20.50 (14-24)	0.07 (0.1)	3.64 (2.6)	—	—
Hoppers Wood, Durham	20	0.45 (0.3)	17.71 (15-22)	0.15 (0.2)	4.92 (2.8)	—	—
Forfar	10	0.70 (0.3)	17.90 (12-22)	0.50 (0.2)	4.50 (3.6)	—	—
Kilmarnock	7	2.43 (2.4)	21.43 (15-25)	0.71 (0.2)	2.14 (0.5)	—	—
Moorhouse Westmorland	15	0.07 (0.1)	19.32 (14-24)	0.07 (0.1)	4.40 (2.7)	—	—

Range is given in brackets.

tetraploid forms, *A. stolonifera* ($2n = 28$) and *A. canina* ssp. *montana* (Melderis, 1955) have been reported. Some odd-numbered polyploids known to be apomictic are occasionally slightly sexual, one of these is the pentaploid ($2n = 35$) *Rubus noveboracensis* (Einset, 1951). However, though there is apparently a high elimination of aneuploids, and these may constitute a high proportion of the seed from an outcross, *Caltha palustris* is a successful species in the wild. The role of vegetative reproduction may be very important in such a situation.

If the $2n = 56$ race is a heptaploid, as we postulate, the rare plants with $2n = 28$ could either be polyhaploids, or aneuploids. Haploids are rare in nature but do turn up occasionally. Aneuploidy is so frequent in *Caltha palustris* that it is more likely that this is the explanation, especially since both counts come from areas where the tetraploid ($2n = 32$) plant is common.

The octoploid plants discovered in North Wales have irregular meiosis, but Reese (1954) has reported that a German race with $2n = 64$ has regular meiosis.

The tetraploid, heptaploid and octoploid races are outbreeders. Chiasmata are often distally localised at metaphase and their number restricted to usually one per chromosome arm. This sort of pairing in outbreeders restricts recombination, but despite the consequent reduction of variation

in populations, gene combinations of possible adaptive value are preserved. The small amount of recombination allowed ensures re-adaptation if habitat changes occur.

The phylogenetic relationships between the three races, $2n = 32, 56$ and 64 , are difficult to determine. There are no clear cytological markers. The heptaploid and octoploid races usually have a pair of satellited chromosomes in their mitotic complements; fewer than their chromosome sets. Darlington (1963) regards this as characteristic of allopolyploids in nature. Probably structural and possibly physiological changes occurring during their evolution have caused some chromosomes originally associated with nucleolar organisation to lose this ability (Schulz-Shaeffer, 1960). It might also be the result of hybridisation, as Navashin's (1934) experiments on *Crepis* indicated.

TABLE 9

Some possible modes of origin of the chromosome races of Caltha palustris

TETRAPLOID	$AA \times BB \dots\dots AB (F_1^*) \dots\dots d \dots\dots AABB (4 \times)$
HEXAPLOID	$AABB (u) \times AABB \dots\dots AAAABB (6 \times)$
or	$AB \times AABB \dots\dots AAB \text{ or } ABB (3 \times) (F_1^*)$
	$AAB \text{ or } ABB \dots\dots d \dots\dots AAAABB \text{ or } AABB (6 \times)$
OCTOPLOID	$AAAABB (u) \times AABB \dots\dots AAAAABB (8 \times)$
or	$AAAAAB (u) \times AABB \dots\dots AAAAABB (8 \times)$
or	$AAABBB (u) \times AABB \dots\dots AAABBBB (8 \times)$
or	$AABB \dots\dots d \dots\dots AAAAABB (8 \times)$
HEPTAPLOID	$AAAAABBB \times AAABBB \dots\dots AAAAABB \text{ or } AAABBB (7 \times)$
or (e.g.)	$AAAAABBB \times AAAABB \dots\dots AAAAABB \text{ or } AAABBB (7 \times)$
	$AAAAABBB \times AAAABB \dots\dots AAAAABB (7 \times)$
or	$AAABBB \times AABB (u) \dots\dots AAAAABB (7 \times)$
or (e.g.)	$AAAAAB \times AABB (u) \dots\dots AAAAABB (7 \times)$
or	$AAABBB \times AABB \dots\dots AAAAB \text{ or } AABBB (5 \times)$
and (e.g.)	$AAABB (u) \times AABB \dots\dots AAAAABB (7 \times)$

(u) = unreduced gamete; d = doubling of number;

(F_1^*) = Sterile F_1 hybrid with *A* and *B* non-homologous.

The only way to be really certain about the status of any polyploid is to resynthesise it from its putative parents. Diploids are rare in *Caltha palustris* and were not available to us. We made repeated unsuccessful attempts to raise artificial octoploids from natural tetraploids, using colchicine. However, we have constructed a tentative scheme to illustrate the possible modes of origin of the chromosome races (table 9).

On the assumption that the tetraploid is an allotetraploid we suggest that the heptaploid will possess four sets of chromosomes from one diploid ancestor and three from the other, and would have had an initial genomic constitution of $AAAABB$ or $AAABBB$ if the contribution of the ancestral diploids is represented by *AA* and *BB*. It would be an autoalloheptaploid. It will be recalled that during meiosis in the heptaploid quadrivalents are fairly common and are usually the highest associations formed. This could be taken as support for the proposed genomic constitutions. The low frequency of univalents and trivalents, and the occasional occurrence of associations higher than quadrivalents may be the result of structural changes between the ancestral genomes.

The octoploid race has high bivalent formation, low quadrivalent number and very low frequency of univalents and trivalents; complete bivalent formation in Continental material. It could be an auto-allooctoploid, with the constitution *AAAABBBB*.

The success of the tetraploid and especially the heptaploid races of *Caltha palustris* may be a result of their hybrid constitution and perennial habit; all have effective rhizomes. More important, each race is fertile and outbreeding and more or less genetically isolated. Isolation is complete between the tetraploid and octoploid races, and almost complete between tetraploid and heptaploid. Further evolution within the species is possible with fuller development of isolation between the races, and also with the apparent ease with which aneuploids can be formed.

(ii) *The distribution of the races*

Unlike many species and species complexes, there is no geographically sharply defined distribution of the cytotypes. The $2n = 32$ race is the most widespread, ranging throughout the Arctic in North America and Europe. It is absent from Britain. The $2n = 56$ race is rare in North America, only two counts of $2n = 56$ having been made there (Borman and Beatty, 1955; Suda, 1969; both in Alaska). In Europe it is widespread, from the U.S.S.R. to Britain and Iceland.

There is evidence in many genera that the climatic changes and migrations consequent upon the Pleistocene glaciations have favoured allopolyploidy. Among such are *Biscutella laevigata* (Manton, 1934); *Crepis* (Babcock and Stebbins, 1939); *Paeonia* (Stern, 1946); *Vicia* (Rousi, 1961) and the Angiosperms of the Ogotoruk creek-Cape Thompson area of N.W. Alaska (Johnson and Packer, 1965). In Europe, the pressures consequent upon the predominantly east-west orientation of the mountain ranges probably resulted in a series of periods of high stress, alternating with times when the retreat of the ice left large areas of unstable, poorly drained base-rich soil suitable for rapid colonisation by species like *Caltha palustris*. It is not surprising that it displays its greatest diversity both of stable chromosome races and of aneuploidy in Europe.

(iii) *Taxonomic considerations*

The taxonomy of *Caltha palustris* *sensu lato* has always been confused; distinctions between the taxa which have been defined are not clear and variation is often continuous. Although populations have minor differences, cultivation experiments show many of them to be habitat imposed and to disappear under uniform conditions. There is no evidence in Britain of major discontinuities in morphology, and we regard all the species and subspecies that have been delimited by others as invalid (Woodell and Kootin-Sanwu, 1971). Evidence from Europe (Smit, 1967) and North America (Hultén, 1968) indicates that in these areas taxonomic differentiation is equally hazardous. Thus, *Caltha palustris* is interesting in that there exist well-defined, partially or completely isolated chromosome races which if morphologically distinct could be regarded as separate species, but which must, on present evidence, be regarded as one. In connection with this, Smit (1967) has detected slight ecological differences between the $2n = 32$ and $2n = 56$ races.

(iv) *Conclusions*

Caltha palustris s.l. is a highly successful species, which has, by polyploidy and hybridisation, established itself in wet habitats throughout the Arctic and Boreal regions. The relative ease with which new chromosome combinations can arise, its obligate outbreeding, and its efficient vegetative reproduction endow it with the means to maintain itself, and at the same time to evolve in response to habitat changes should they occur.

6. SUMMARY

1. *Caltha palustris* L. s.l. is sexually reproducing and self-incompatible. Pollen fertility of all races is high, but elimination of unbalanced forms occurs at the seedling stage.

2. Crosses between the $2n = 32$ and $2n = 56$ races produce progeny with numbers ranging from $2n = 42$ to 45, about 75 per cent. having $2n = 44$. All are pollen sterile. The $2n = 56 \times 2n = 64$ crosses produce offspring ranging from $2n = 56$ to 63. Meiosis is very disturbed in these hybrids. The $2n = 64$ and $2n = 32$ races fail to cross. Crosses within the races are fully fertile.

3. The irregular meiosis, the basic number of 8, the large number of aneuploids and the frequency of unreduced gametes under experimental conditions indicate that the $2n = 56$ race is a heptaploid. No taxonomic differentiation can be made on the basis of chromosome number.

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