# Morphological and cytological studies on a hexaploid clone of *Potentilla anserina* L.

### A. M. COBON and B. MATFIELD

Department of Botany and Biochemistry, Westfield College, London University

# ABSTRACT of the of goining open ni say shift to protected

A study was made of the morphological characters and meiotic behaviour of a single clone of hexaploid *Potentilla anserina* (2n = 6x = 42). Observations on leaf, epicalyx, and pollen characters confirmed previous results. The hexaploid cytodeme is morphologically very similar to the tetraploid, suggesting that it may be derived from it. The hexaploid behaved at meiosis like an autotriploid, forming a high proportion of trivalents at metaphase I. The tetraploid regularly forms bivalents, and may be an allopolyploid. Hexaploid *P. anserina* could, therefore, have an autoallopolyploid origin, arising possibly from the fusion of reduced and non-reduced tetraploid gametes.

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Potentilla anserina L. is a well-known British species with a wide geographical distribution. Rousi (1965) made an extensive study of the species aggregate and summarized earlier taxonomic treatments and chromosome counts. Three different chromosome numbers have been reported, 2n = 4x = 28, 2n = 5x = 35, and 2n = 6x = 42. Rousi determined the chromosome numbers of plants from 47 different localities in Europe, N. America, and New Zealand, finding 44 tetraploids and three hexaploids, which came from California, Finland, and York, England. The last was Rousi's only British specimen, which suggested that the frequency of hexaploids in Britain might be higher than elsewhere. However, Ockendon & Walters (1970) made chromosome counts of British specimens from 31 localities and found only four hexaploids, one of which came from the same place as Rousi's hexaploid. The others occurred in Cambs., v.c. 29, Caerns., v.c. 49, and Bute, Clyde Isles, v.c. 100. The results of both investigations suggested that the hexaploids have a polytopic origin from the tetraploids.

Rousi (1965) examined 19 different morphological characters and found no significant difference between tetraploids and hexaploids in the majority of them. The only vegetative character that seemed potentially useful in distinguishing between the two races was the length/breadth ratio of the terminal leaflet. This was lower in all three hexaploids than in tetraploids from the same or neighbouring areas. Ockendon & Walters looked at three of Rousi's characters: length/breadth ratio and tooth number of the terminal leaflet, and form of the epicalyx segments. They concluded that 'the two cytodemes cannot be distinguished with absolute certainty by any character other than the chromosome number'.

In his study of meiosis in tetraploids from four sources, Rousi found that 'metaphase I is usually quite regular, the 14 bivalents being well orientated. No multivalents were found in the tetraploids'. He also examined meiosis in his British hexaploid and reported that 'diakinesis was characterized by a surprisingly low number of distinct bodies, either 14 or a few more'. However he considered a high frequency of trivalents unlikely since 'in general plants with small chromosomes seldom have a high number of pairing blocks and chiasmata.' He suggested the possibility of secondary pairing between univalents and homologous bivalents, and noted that anaphase I and later stages showed numerous irregularities.

This paper is concerned with a morphological and cytological investigation of a hexaploid clone of *P. anserina* found beside the tow-path of the Union Grand Canal near Tring, Herts., v.c. 20 (GR 42/924.138). It was morphologically indistinguishable from other tetraploid specimens collected in the same locality. Only subsequently was its ploidy level discovered, from chromosome preparations made after a period of cultivation in an experimental garden.

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#### METHODS AND RESULTS

#### MORPHOLOGY AND FERTILITY

#### Length/breadth ratio of terminal leaflet

Rousi determined this ratio in plants from 20 different sources. The mean values for individual plants ranged from 1.51 to 3.35. The three hexaploids all had low ratios, from 1.51 to 2.06, but several tetraploids also had ratios lower than 2.00. Ockendon & Walters confirmed Rousi's findings; their three hexaploids all had low ratios of between 1.6 and 2.0 but the ratios for tetraploids ranged from 1.5 to 3.1. In our hexaploid the length/breadth ratio of the terminal leaflet was 2.4 (mean of ten leaves), a higher value than previously reported for a hexaploid. This character is therefore of little use in recognizing hexaploids in the field.

#### Number of leaflet teeth

On his sample of tetraploids and hexaploids, Rousi counted the number of teeth on one side of the terminal leaflet, including the terminal tooth, and found a range of 7.0 to 13.8. The range given by Ockendon & Walters was 7 to 13. There was no correlation between number of teeth and ploidy level. Our hexaploid had an average tooth number of 11.8 (ten leaves).

#### Epicalyx segments

Rousi's British hexaploid from York had laciniate epicalyx segments, and Ockendon & Walters confirmed that plants from this population had larger, more deeply toothed epicalyx segments than most of the tetraploids which they examined. Our hexaploid similarly had long laciniate segments. However, Rousi warned that this character is inconsistently expressed in many populations and is not, therefore, a good indicator of ploidy level.

#### Pollen grain stainability and size

Pollen fertility and grain size are directly affected by meiotic irregularities and these characters are therefore most useful in identifying polyploids or hybrids. Rousi measured pollen stainability in cotton blue for the tetraploids (from 19 different sources) and found a range of 59.8 to 98.1% whilst the three hexaploids had from 18.9 to 22.2%. Ockendon & Walters did not find such a clear difference; their hexaploids all had low levels of pollen stainability, ranging from 2 to 28%, but several of the tetraploids had unusually low values down to 26%. They concluded that plants with values of 25 to 35% could be tetraploid or hexaploid. They also noted that the value for a single plant varies widely at different times of the year.

The pollen of our hexaploid was stained with acetocarmine and the percentage of stainable pollen was 29.7%, the highest value so far recorded for a hexaploid. A tetraploid (growing in an adjacent pot) which was sampled on the same day gave a rather low value of 50.6%. This suggests that the relatively high value for the hexaploid was not due to especially favourable environmental conditions.

Rousi measured the size of stainable pollen grains and found that his three hexaploid populations had larger mean values than most tetraploids, although two tetraploid populations had larger

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	13	ind roled (	ous birfilents, a	30	25.2
	12	2	2	24	20.2
	to noll silver	aile 3 nint	vo bo 3 mol	14	11.8
	10	4	4	8	6.7
	8	6	6	ibni 1 mino	0.8
				119	

#### TABLE 1. CHROMOSOME CONFIGURATIONS AT DIAKINESIS AND METAPHASE I IN HEXAPLOID P. ANSERINA

#### HEXAPLOID POTENTILLA ANSERINA L.

values than the hexaploids. More significant was the variability shown by the hexaploid grains. Even when empty grains were excluded, the hexaploid pollen grains were much more variable in size than those of the tetraploids. This was confirmed in our own plants: the average pollen grain diameter for the hexaploid was  $30.1\mu$ m with a range of  $20.0-37.5\mu$ m. The tetraploid plant had much more uniform grains with a mean diameter of  $27.4\mu$ m and a range of  $25.0-28.7\mu$ m.

# Achene formation

Rousi's hexaploids did not produce any achenes after artificial self- or cross-pollination. The tetraploids were seed-fertile but completely or partially self-incompatible. Our hexaploid formed no achenes, but, as only a few plants of either cytodeme were cultivated, this may have been due to a lack of compatible pollen.

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Buds were fixed in freshly prepared acetic alcohol (acetic acid: ethanol, 1:3) at about  $10^{\circ}$ C for a minimum period of a week. Anthers were squashed in strong acetocarmine solution and the stain was finally replaced by 45% acetic acid to prevent excessive colouration of the cytoplasm. Cells were observed under a ×100 oil immersion phase-contrast objective.

#### Meiotic observations

A total of 119 pollen-mother-cells was examined at diakinesis or metaphase I and their chromosome configurations are summarized in Table 1. The most striking feature was the high trivalent frequency; the configurations were clearly trivalents and not secondary associations between



ancient phenomenon in the group. An increase in obtomosome number from 4x to 6x could be achieved in a single step by the fusion of reduced and non-reduced gametes. Functional non-reduced gametes have been demonstrated in related species. Munizing (1958) reported the occasional fertilization of non-reduced egg cells in hexaploid *P. collina* Wibel, giving rise to nonaploid individuals. Unreduced gametes are also by the related requester of the closely related control the evolution individuals. Unreduced gametes are also by the evolution of the closely related control the evolution of the closely related control the evolution.

FIGURE 1. Two metaphase I chromosome configurations in hexaploid *P. anserina*: A) 14 trivalents; B) 13 trivalents, 1 bivalent (double arrow), 1 peripheral univalent (single arrow).

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bivalents and univalents as suggested by Rousi. The maximum possible trivalent number in a cell with 42 chromosomes is 14 and this was observed in 35% of the cells (Fig. 1A). The mean trivalent number was 12.6 and the lowest was 8. The univalents were usually around the periphery of the cell (Fig. 1B). The bivalents and trivalents were aligned on the metaphase plate, and were often distinguishable only by their different sizes. Potentilla chromosomes are too small for the number and positions of chiasmata to be accurately determined. However, the rarity of protruding chromosome arms indicated that chiasmata were generally terminal at diakinesis. Some of the trivalents were clearly V- or 'pan-handle'-shaped (Fig. 1B). Where the three chromosomes were arranged in a chain, its metaphase orientation was usually convergent rather than linear. Many of the trivalents were very compact and asymmetrical; although three chromosomes were clearly present, their exact arrangement was obscure. At anaphase I and subsequent stages disjunction was uneven and univalents were often excluded from the polar groups and formed micronuclei. or occasionally were left at the equator. Such univalents divided into chromatids as they normally would at anaphase II. In two cells it was possible to count accurately the number of chromosomes at each pole; in one the groups contained 19 and 23 chromosomes, and in the other 20 and 22 chromosomes. The latter cell contained one pair of chromosomes still associated at late anaphase I.

#### DISCUSSION

The lack of any clear morphological distinction between tetraploid and hexaploid *P. anserina* was observed by Rousi (1965) and Ockendon & Walters (1970), and is confirmed by the present study. In *Potentilla*, an increase in ploidy level is usually accompanied by a series of morphological changes including a decrease in the length/breadth ratio of leaves and petals (Matfield 1968). The higher polyploid is sometimes, but not always, gigas. When Matfield (1968) doubled the chromosome number of two tetraploid *Potentilla* species, *P. erecta* (L.) Räusch. and *P. reptans* L., by colchicine treatment, the octoploids of *P. reptans* had larger leaves than the tetraploids, whereas the reverse was the case in *P. erecta*. In both species the decrease in length/breadth ratio of the leaves was, however, very marked. Although the comparison in *P. anserina* is between tetraploid and hexaploid it is surprising that the morphology of the two cytodemes is so similar. This similarity between hexaploid and tetraploid *P. anserina* species aggregate and Rousi concluded that tetraploidy is an

4 63 20		No. of trivalents		% of chromosomes	
	x	range	mean	as trivalents	Reference
Dactylis glomerata L.	7	1–7	4.7	56.7	Carroll (1966)
Anthoxanthum ovatum Lag.	5	0-5	3.2	63.5	Carroll (1966)
Tulipa praecox Ten.	12		10.2	85.0	Upcott (1939)
Potentilla erecta (L.) Räusch.					100
(6x - see text)	7	8-14	12.3	87.6	Matfield & Ellis (1972)
Impatiens balsamina L.	7	3–7	6.2	89.3	Smith (1938)
Tradescantia bracteata Small	6		5.4	90.0	King (1933)
Zea mays L.	10	6–10	9.2	92.4	Punyasingh (1947)

#### TABLE 2. MEIOTIC BEHAVIOUR IN AUTOTRIPLOIDS

ancient phenomenon in the group. An increase in chromosome number from 4x to 6x could be achieved in a single step by the fusion of reduced and non-reduced gametes. Functional non-reduced gametes have been demonstrated in related species. Müntzing (1958) reported the occasional fertilization of non-reduced egg cells in hexaploid *P. collina* Wibel., giving rise to nonaploid individuals. Unreduced gametes are also believed to have played an important part in the evolution of the closely related genus *Fragaria* (Ellis 1962).

Tetraploid *P. anserina* forms bivalents regularly at meiosis (Rousi 1965). This suggests that the tetraploid could have an allopolyploid origin, which may be represented genomically as AABB.

The high frequency of trivalents in the hexaploid suggests that it is probably an autoallopolyploid, which may be represented as AAABBB, and which behaves cytologically as an autotriploid.

For comparison with the meiotic behaviour of hexaploid *P. anserina*, Table 2 shows the trivalent frequencies in a number of known autotriploids. Most of them have high trivalent frequencies, with about 89% of the chromosomes taking part in trivalent configurations; the two grasses studied by Carroll (1966) had somewhat lower multivalent frequencies. The hexaploid *P. erecta* in Table 2 was artificially produced from the naturally occurring tetraploid (Matfield & Ellis 1972). It is included because, as in *P. anserina*, the tetraploid has regular bivalent formation in meiosis, indicating a possible allopolyploid origin. Hexaploid *P. erecta* has, therefore, like hexaploid *P. anserina*, a probable autoallopolyploid origin, and behaves cytologically like an autotriploid.

It can be seen that the trivalent frequency in hexaploid *P. anserina* is as high as that in a number of other species, despite the small size of *Potentilla* chromosomes. It is usually thought that autopolyploids with small chromosomes form fewer multivalents than those with larger chromosomes. This follows from Darlington & Mather's (1932) theory that small chromosomes contain fewer 'pairing blocks'. Although this generalization may hold true for chromosomes of different sizes within the same complement, it does not seem to apply to differences of size between unrelated species. Morrison & Rajhathy (1960) came to the same conclusion when they compared quadrivalent frequencies in ten different autotetraploids, and found that as many quadrivalents were formed in species with small chromosomes as in species with larger ones.

In tetraploid *P. anserina* the relative importance of vegetative and sexual reproduction is unknown. Flower production in vigorous wild populations is often poor, and runners of up to one metre in length may be produced in a single growing season (Ockendon & Walters 1970). The low fertility of the hexaploids may therefore result in no disadvantage in short-distance dispersal, although Ockendon & Walters suggested that it may prevent them from spreading far from their point of origin. However, the plant used in this study was found on a canal tow-path, a habitat in which opportunities for the dispersal of detached vegetative parts must be good.

#### ACKNOWLEDGMENTS

We are grateful to Dr B. Murray of Westfield College for his encouragement and his critical reading of the manuscript.

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(Accepted November 1975)