# STUDIES ON ALCHEMILLA FILICAULIS BUSER, SENSU LATO AND A. MINIMA WALTERS.

## II. CYTOLOGY OF A. FILICAULIS, SENSU LATO

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#### Abstract

All chromosome counts of segregates in the Alchemilla vulgaris L. aggregate are listed. Cytological preparations of root-tips, grown in water-culture, and pollen-mother-cells of A. filicaulis, sensu lato, were examined with a phase-contrast microscope. It was difficult to obtain exact counts; in both subspecies 2n = c. 101–110, and one count of a dwarf montane plant (see p. 323) gave 2n = c. 105  $\pm$  2. P.M.C.'s stained more easily, but the small and variable number of probable bivalents made interpretation difficult. One plant of A. filicaulis had 2n = c. 150.

#### INTRODUCTION

Cytological studies of the segregates in the *Alchemilla vulgaris* L. aggregate have proved very difficult. Although the chromosomes are small it was difficult to spread the large number sufficiently well to obtain accurate counts. Löve & Löve (1961) list the counts obtained so far within the *A. vulgaris* aggregate. All are high polyploids and apomicts. The particular type of embryology will be described in the third paper of this series.

Turesson's counts (1957) of the A. vulgaris L. agg. appear to be the most accurate, but he was not able to give absolutely exact figures, each value being within range of the figures given. However, the accuracy is sufficient to show that some species have a number of 2n = c. 105–106; these are : A. cymatophylla Juz., A. sarmatica Juz. and A. xanthochlora Rothm. Most of the others probably have several numbers between 2n = 100 and 110; these are : A. glaucescens Wallr. (A. minor Huds.), A. acutiloba Opiz, A. subcrenata Bus., A. gracilis Opiz., A. subglobosa C. G. West, A. vestita (Bus.) Raunk., A. filicaulis Bus., A. monticola Opiz., A. glabra Neyg., A. obtusa Bus., A. wichurae (Bus.) Stef., A. murbeckiana Bus. and A. glomerulans Bus. The lower values of 2n = 93 and 96 are undoubtedly counts of insufficiently spread plates, the latter an obvious multiple of 8 which has been considered to be the basic number.

A higher range of numbers is found in *A. oxyodonta* Bus. (2n = c. 165-168), *A. os-karssonii* Löve (= *A. glacialis* Oskarss.) (2n = c. 144) and *A. transpolaris* Juz. (= *A. borealis* Sam.) (2n = c. 130-152).

In addition to the chromosome numbers given for A. wichurae, A. murbeckiana, A. oxyodonta and A. glomerulans, the value 2n = c. 64 is reported by Löve & Löve (1956) and for the last by Bocher (1938) also. Differences of this magnitude are unlikely to be errors in counting, so that if the material has been identified correctly they imply the existence of intraspecific chromosome races. There may be some significance in the fact that

Turesson's material was of Scandinavian origin whilst Bocher's was from Greenland and Löve & Löve's was from Iceland; though the material of Jørgensen *et al.*, was also from Greenland. Obviously some rechecking is needed here.

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#### Method

Preparations of root-tips and pollen-mother-cells were made, and both had their special difficulties. Cells of the root-tips seemed to divide rarely; the chromosomes were difficult to spread and stained poorly. The pollen-mother-cells were easier to handle and stained better, but were more difficult to interpret than the root-tip preparations.

The best results were obtained from roots grown by a simple water-culture method. All except the young roots were stripped from the rhizome of a healthy shoot before it was suspended in a beaker of water. When new adventitious roots had grown, after a few weeks, several millimetres of the yellow-tipped roots were cut off and pre-treated with 0.002 M 8hydroxyquinoline. The tubes were immersed in running water ( $c.15^{\circ}$ C) for 4 hours. Root-tips were fixed in acetic-alcohol, placed in a domestic refrigerator (c.  $4^{\circ}$ C) for 3-6 days to increase the spread of the chromosomes (Walker 1956), and then stored in a deep-freeze refrigerator. It proved difficult to get well spread, deeply stained chromosomes; best results were obtained with aceto-carmine stain using a modification of the combined staining and maceration technique of Proctor (1955). The root-tip was heated in about 1 cc of an aceto-carmine - N HC1 mixture in the proportions 9:1 in a test-tube in a boiling water-bath for 5-7 min. Iron was introduced by standing a cleaned needle in the test-tube or earlier as a few drops of ferric acetate in the fixative. The hydrochloric acid aided maceration and hence helped to increase the spread of the chromosomes but had the disadvantage of reducing the stain uptake by the chromatin material. However, better definition was obtained by the use of a phasecontrast microscope.

For meiosis flower buds were fixed when the inflorescences were still very compact, preferably in April, though flowering continues until September or even later. The flowerbuds were separated and fixed in acetic-alcohol and chloroform (1:3:1) for at least 24 hr, then stored in 70% alcohol in a deep-freeze.

Meiosis takes place when the flower buds are about 2 mm long. The four anthers were dissected out into a drop of aceto-carmine, split, and as much of the wall as possible removed. At the correct stage for metaphase plates the pollen-mother-cells float out as separate round cells. These were stained in the usual way. Only a small drop of aceto-carmine must be used or the PMC will float to the edge of the cover-slip and be lost; more stain can be added from the edge of the cover-slip if air gaps remain.

Suitable preparations were made permanent in Euparal. All the chromosome numbers were high (2n = c. 100). To obtain accurate counts, an enlarged photographic print was used as a base on which to ink-in the chromosomes as each was observed under the microscope. In this way, no chromosome is missed or duplicated, and any uncertainty about the exact number is due to inability to interpret the preparation. This method is described by Manton (1950).

## Results

The high number of chromosomes, and the difficulty of getting them well spread, made it almost impossible to obtain exact counts; somatic counts from the root-tips are given below.

Subspecies	Code No.	Locality and grid ref.		2 <i>n</i>		
vestita	4/9/101	Teesdale	35/921273	$105\pm2$		
				$   \begin{array}{r}     105 \pm 2 \\     104 \pm 2   \end{array} $		
vestita	4/24/300a	Teesdale	35/861296	$104 \pm 1$		
				$105 \pm 2$	(Plata 14a)	
				$100 \pm 1$ $101 \pm 1$	(Flate 14a)	
				$101 \pm 1$ $106 \pm 1$		
vestita	4/39/269a	Teesdale	35/865309	$104\pm2$		
				$104 \pm 1$		
				$105 \pm 1$		
	4/20/2106	Waandala	25/000270	$104 \pm 1$	$(\mathbf{\Gamma}^{1}, 1, 1)$	
vestita	4/30/3180	weardale	35/9003/8	106 exact	(Fig. 1a)	
vestita	5/45/250a	Mickle Fell	35/815247	$105 \pm 2$		
(montane)	2 ( ( 2)					
filicaulis	3(60) n	Meall nan	27/5838	$152 \pm 3$		
	,, m	Tarmachan	**	$150 \pm 2$	(Fig. 1d)	

<sup>(</sup>a) Mitotic counts

The probable margin of error is given in each case.

The count for 4/30/318b is thought to be exact.

### (b) Meiotic counts

Preparation of pollen-mother-cell squashes was relatively easier than the root-tips and stain differentiation was better, but the interpretation was much more difficult. In all cases there were many univalents, and it was not easy to determine which were bivalents or even if multivalents were present; no certain cases of the latter were found. Certain closely associated pairs of chromosomes have been queried as probable bivalents; whether they are interpreted as such or regarded as univalents makes no difference to the total chromosome number. All interpretations are made in the light of somatic counts obtained from the root-tips. In general, when the larger chromatin bodies were regarded as bivalents and the smaller as univalents, the total chromosome number obtained was near the somatic value of the species; thus this interpretation seems justified and does give some idea of the number of bivalents which usually occur. It is essential to know the somatic value when studying meiotic figures as other interpretations could easily be made; because of this all chromosome numbers are approximate only.

Subspecies	Code No.	Locality and grid	2 <i>n</i>		
vestita	4/2/31 4/45/414b	Weardale E. Durham	35/963392 35/338384	109? c. 108	
filicaulis	4/3/55a	Weardale	35/964392	<i>c</i> . 103 <i>c</i> . 103	
filicaulis	3(60)b	Meall nan Tarmachan	27/5838	c. 150	

Each plate will be considered separately. In the explanatory diagrams, the univalents are outlined, the bivalents and closely associated chromosomes are blacked in.

subsp. vestita 4/2/31 (Fig. 1c). This was very well squashed and is probably a late anaphase, though it could be regarded as a metaphase II. All the chromatin bodies are much the same size and if interpreted as single chromosomes give 2n = 109 or 110; which is near the number obtained for the somatic counts in the species and the same as that given for *A. vestita* by Turesson.



Fig. 1. Chromosomes of Alchemilla filicaulis, sensu lato. (a) subsp. vestita, Weardale (RT, 2n = 106,  $\times$  770): (b) subsp. filicaulis, Weardale (PMC, 2n = 103,  $\times$  530); (c) subsp. vestita, Weardale (PMC, 2n = 109 or 110,  $\times$  1070) (d) subsp. filicaulis, Meall nan Tarmachan (RT,  $2n = 150 \pm 2$ ,  $\times$  760); (e) subsp. filicaulis, Meall nan Tarmachan (PMC, 2n = c. 150,  $\times$  1000). Doubtful bivalents are marked ?b. For further explanation see text.

subsp. vestita 4/45/414b (Plate 14b). This is a metaphase plate with 21 II and 66 I giving 2n = 108.

subsp. *filicaulis*. The only somatic count of British material is of somewhat atypical material 3(60) which is considered separately below. Turesson has published values 2n = 101 to 110 for Scandinavian plants. Interpretation of the meiotic figures of British material has been based on the assumption that this has approximately the same range of numbers.

subsp. *filicaulis* 4/3/55a (Plate 14c). A fairly straight forward metaphase I with 20 II and 63 I giving 2n = 103. Same plant (Fig. 1b) also a metaphase I plate with 2n = 103: 18 II and 67 I. Although the number of bivalents varies the total number of chromosomes is 2n = 103; it is of particular interest to know if the somatic count is the same, but a countable preparation was not obtained.



Chromosome plates and explanatory diagrams of Alchemilla filicaulis, sensu lato. (a) subsp. vestita, Teesdale (RT,  $2n = 106 \pm 1$ ,  $\times 1340$ ); (b) subsp. vestita, E. Durham (PMC, 2n = c. 108,  $\times 1310$ ); (c) subsp. filicaulis, Weardale (PMC, 2n = c. 103,  $\times 1020$ ). Doubtful bivalents are marked ?b. For further explanation see text.

subsp. *filicaulis* 3(60)b (Fig. 1e). The presence of the nucleolus and indication of an outer membrane suggest that this is at diakinesis or maybe metaphase I. The diffuse nature of the chromatin bodies makes their interpretation as univalents or bivalents difficult and hence the total number uncertain. Two clusters A and B probably consist of a total of 6 or 7 chromosomes. Of the other bodies, if the larger are interpreted as bivalents, the probable values are 20 II and 117 I giving a total of 159; if all are considered as univalents the total is 144, one above and the other below the somatic value of 2n = c. 150.

## (c) Cytology of subsp. vestita.

The mean values obtained for lowland plants of subsp. vestita were :

Somatic counts 2n = 104, 105, 105, 106.

Meiotic counts 2n = c. 108, c. 109.

In spite of the closeness of the somatic numbers and the difficulty of getting exact counts, it is very probable that several chromosome numbers do occur in the species. Turesson (1957) obtained a range of numbers between 101 and 110 for several species of *A. vulgaris* agg. He gave 2n = 110 for the only two plants of ssp. *vestita* which he examined.

A count was obtained for only one of the dwarf montane plants; this gave 2n = 105, which is within the range of the lowland plants.

### (d) Cytology of subsp. filicaulis

The only count obtained of a British lowland plant was from a pollen-mother-cell, 2n = c. 103. Turesson (1957) found a range of numbers :

2n =	102	103	104	105	106	107	108	109	110
No. of plant	ts 2	6	1	7	5	1	3	4	2

This overlaps the range found in subsp. vestita.

Most interesting and surprising is the value 2n = 150-152 obtained for a subsp. *filicaulis* plant, 3(60), which represents a Scottish mountain population. These plants have the usual subsp. *filicaulis* hair character and wine-red base, but the leaves have very short broad lobes and sharply pointed, somewhat connivent teeth, rather similar to A. wichurae; the leaves tend also to be more shiny and crisp than in other subsp. *filicaulis* plants. They could easily be recognised in the field from amongst plants of A. vulgaris agg. Whether or not this plant should be classified as subsp. *filicaulis* will be considered later.

The chromosome numbers obtained by Turesson (1957) and myself for subsp. *vestita* and subsp. *filicaulis* agree well and show no major differences in the cytology of the two taxa except in the case already mentioned. Both have a range of numbers which are similar to those found in several distinct morphological species in *A. vulgaris* agg. (Turesson 1957). The difference in hair density which separates subsp. *filicaulis* from subsp. *vestita* is not correlated with any constant difference in the chromosome numbers of these taxa.

So far no counts are available for the genodeme of intermediate hairiness.

### (e) Cytology of the ecogenodemes

A chromosome count of one example of the dwarf montane ecogenodeme (Bradshaw 1963, p. ) is clearly an insufficient basis for any conclusions on the correlation between chromosome numbers and 'ecotypic' variation. If it is representative of the dwarf ecogenodeme then there would appear to be no major cytological differences between the ecogenodemes. Further data on the same kind of variation are provided by Turesson (1956). He worked with six species (but not *A. filicaulis*), and also found statistically significant differences in stem and petiole length and leaf size between genodemes. Later (1957) he published chromosome counts of many species but it is not clear if the numbers are those of the plants used in the earlier work or not. Turesson did not give the chromosome numbers of his morphological genodemes individually; neither is it known if the plants used in his experiment

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all came from the same or different types of habitat. Turesson concluded 'Our Alchemillas apparently do not adapt themselves to different habitats by any change in chromosome numbers.' Difference in habitat is implied by the wide latitudinal range of the original samples. Only by obtaining exact counts of several plants of each genodeme will it be possible to determine if this kind of variation is correlated with differences in the chromosome numbers.

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