Chromosome Studies in *Anemone* I. A New Type of Chiasma Behaviour

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1. Introduction

CHIASMA formation and terminalisation at meiosis have now been studied in several groups of plants. The evidence accumulated so far shows two main types of behaviour.

(1) Terminalisation is complete, resulting in all or the great majority of the chromosomes being attached by terminal chiasmata at metaphase of the first reduction division, as shown in *Primula sinensis* (Darlington 1931) and as may be inferred in *Datura Stramonium* (Belling and Blakeslee 1923).

(2) Terminalisation is not complete and the chromosomes at metaphase I are attached by a variable number of interstitial chiasmata, e. g. *Fritillaria* (Darlington 1930) *Pisum* (E. Richardson Sansome 1931) *Stenobothrus* (Darlington and Dark 1932). Usually where there has been a decrease in the number of chiasmata between prophase and metaphase, terminal chiasmata are found at metaphase. In *Stenobothrus* the chiasmata at metaphase are mostly interstitial and it has been observed that there is no decrease in the number of chiasmata per bivalent between diplotene and metaphase—a condition usual in organisms with a low degree of terminalisation.

In *Anemone* the terminalisation appears to be of an anomalous type. There is a considerable decrease in the number of chiasmata per bivalent between diplotene and metaphase I, but a low proportion of terminal chiasmata have been observed at metaphase. The reduction in the number of chiasmata appears to be due to terminalisation followed by the slipping off or breaking of the terminal chiasmata at stages between diplotene and metaphase. In the present paper a statistical study has been made of chiasma formation and terminalisation, to demonstrate these facts. In this paper only diploids will be considered.

2. Material and Methods

Most of the material was obtained from a collection of plants growing at the John Innes Horticultural Institution. Whole buds were fixed in Carnoy's fluid for half a minute followed by La Cour's N2BD. Root tips were fixed in 2BE (La Cour 1930). Pollen mother-cells were cut at 25μ and root tips at 20μ . The fixations gave very variable results in pollen mother-cells. Drawings were made with a 1.5 mm. Zeiss Apochromatic objective and $\times 30$ compensating ocular, with the aid of an Abbé camera lucida. All drawings have been reduced in size to the given magnification.

3. Somatic Numbers

The following chromosome numbers were found in root tips and pollen mother-cells.

Diploids	n	2n
<i>Anemone Hepatica</i> L.	—	14 (28)*) (Fig. 2)
<i>baicalensis</i> Turcz.	8	16
<i>sylvestris</i> L.	8	16
<i>caroliniana</i> Walt.	8	16
<i>borealis</i> Richards	8	16
<i>fulgens</i> Gay	—	16
<i>vernalis</i> L.	—	16 (32)*)
<i>sibirica</i> L.	8	16
<i>japonica</i> S. et Z.	8	16 (Fig. 1)
<i>parviflora</i> Michx.	8	16
<i>mexicana</i> H. B. K.	8	16
<i>vitifolia</i> Buch.-Ham. ex DC.	—	16

*) Diploid and tetraploid cells found in the same root-tips.

Triploids		
	n	2n
<i>Anemone baldensis</i>	24/2	24
<i>decapetala</i>	24/2	24
<i>sylvestris</i> × <i>multifida</i>	24/2	24
Tetraploids		
<i>Anemone magellanica</i> Hort	—	32
<i>rupicola</i> Camb.	—	32
<i>multifida</i> Poir.	16	32
<i>blanda</i> var. <i>rosea</i> Schott & Kotschy	—	32
<i>Pulsatilla</i> Zinn.	16	32
<i>montana</i> Hoffe "B"	16	32
<i>nemorosa</i> Linn.	—	32
Pentaploid		
<i>Anemone nemorosa</i>	—	39
Hexaploids		
<i>Anemone montana</i> "A"	24	48

Some of these numbers agree with those found by previous authors (Takamine 1916, Winge 1917, Langlet 1927). Langlet reports *A. montana* to be diploid. Of the two plants examined here one ("B") was tetraploid and the other ("A") was hexaploid. *Anemone nemorosa*, reported by Winge (1917) to be diploid with a basic number of 6, was found to be tetraploid with a basic number of 8. One form had a chromosome number of $2n=39$ being apparently pentaploid minus one chromosome.

Two of the triploids, *A. decapetala* and *A. baldensis*, obviously cannot be original species. These forms have probably arisen from a cross between a diploid and a tetraploid.

Somatic doubling occurs frequently in two species, *A. Hepatica* and *A. vernalis*, diploid and tetraploid cells being often found in the same root tip.

The somatic chromosomes are fairly large, being about 6μ in length. In *Anemone Hepatica* with 14 chromosomes, one pair has a sub-terminal constriction and a large trabant. The others have median or sub-median constrictions. The chromosome types are very similar throughout the species with 16 chromosomes. Four pairs have median or sub-median constrictions; in one pair the short arm is about $1/3$ the length of the long arm, and there are three pairs with sub-terminal constrictions. One of the pairs with a sub-terminal constriction has a

trabant, but this is considerably smaller than that observed in *A. Hepatica*. The difference between *A. Hepatica* and the rest can be explained as being due to fusion. The range in size of the chromosomes is not very great, the longest being 7μ and the shortest 5μ . Owing to the prevalence of bivalents of the cross type at metaphase it is difficult to distinguish these chromosome types at meiosis.

4. Chiasma Formation and Terminalisation in Diploids

Chromosome pairing and chiasma terminalisation have been studied at all stages of meiosis from diplotene to metaphase. The best preparations were obtained in *Anemone baicalensis*, and this species has been examined in the greatest detail. In the other diploid species examined the sequence of stages closely followed that described in *A. baicalensis*, and a description of meiosis in this species alone shows how terminalisation takes place in the diploids.

Fig. 1. Somatic plate from root tip of *A. japonica*. $2n=16$. $\times 4000$. Four pairs of chromosomes with median or sub-median constrictions. Four pairs with terminal or sub-terminal constrictions.

Fig. 2. Somatic plate from root tip of *A. Hepatica*. $2n=14$. $\times 4000$. Six pairs with median or sub-median constrictions. One pair with sub-terminal constrictions.

At pachytene the paired chromosomes form thick twisted threads in which side-by-side pairing of the chromosomes may be clearly observed. In many cells it was possible to trace out the eight paired chromosomes, and there was no evidence of a "continuous spireme" stage. The chromosomes lie side by side at this stage and as far as could be observed are not twisted about one another to any great extent. There is no evidence of any torsion between the chromosomes (Fig. 3). This stage has not been illustrated completely in a plant

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8.

Figs. 3-8. Stages of Meiosis from diploid *Anemone baicalensis* showing a parallel reduction in the numbers of total chiasmata per bivalent and terminal chiasmata per bivalent from diplotene to metaphase. Fig. 3, eight paired chromosomes from pachytene. Fig. 4, chromosomes opening out after pachytene to form diplotene loops. Note the terminal attachments at this stage. Fig. 5, middle diplotene. Fig. 6, late diplotene. Fig. 7, diakinesis. Fig. 8, metaphase.

before, except in *Bellevallia* (Dark, cf. Darlington 1932), or in any animal except *Dendrocoelum* (Gelei 1922).

The paired chromosomes then begin to fall apart to form loops, which gradually spread along the chromosomes between the "nodes", or chiasmata between the chromosomes. At this stage the chromosomes appear to be longer and more slender than at pachytene (Fig. 4). Such a condition may be an artefact due to fixation, but it is possible that the greater length after pachytene may indicate the release of an internal torsion within the chromosomes, brought about by chiasma formation. The chromosomes are however much twisted both at pachytene and immediately afterwards, and it is difficult to obtain any estimate of their relative lengths. Immediately after pachytene it is not always possible to differentiate between chiasmata and chromosomes twisted round one another, but at this stage the paired threads rarely open out to leave free ends, thus giving from the earliest observable stage what appear to be terminal chiasmata (Fig. 4). These terminal attachments may be caused by collapse of the ends into each other owing to fixation, but the regularity with which they occur seems to indicate that they are terminal chiasmata, and that terminalisation begins immediately the loops start to open after pachytene (as is shown by the fragments of *Fritillaria* (Darlington 1930) and observations of *Tulipa*—Darlington and Janaki-Ammal 1932).

At a slightly later stage when the chromosomes have completely fallen apart between the points of change of partners to form the diplotene loops it was possible to count the number of chiasmata per bivalent. At this stage, which will be referred to as middle diplotene (Fig. 5), owing to the length and twisting of the chromosomes it was only possible to count the complete eight bivalents in one cell. Other configurations were counted at random from different cells. As an indication of the amount of terminalisation which has taken place the total number of chiasmata per bivalent and the number of terminal chiasmata per bivalent have been estimated at various stages. The terminalisation coefficient, i.e. the proportion of the terminal chiasmata to total chiasmata, which has previously been used for comparison, here gives no indication of the number of chiasmata which are terminal at any one stage.

At middle diplotene, the mean total of chiasmata per bivalent was 6.1 and the mean number of terminal chiasmata 1.15 (the maximum possible being 2) indicating that a considerable amount of terminalisation has already taken place. At late diplotene the mean total

chiasma frequency per bivalent is 3.2, and the mean terminal chiasma frequency is .66 (Fig. 6). There is a corresponding decrease in the numbers of total and terminal chiasmata per bivalent until metaphase (see Table 1) (Figs. 7-8).

Table 1

	No. of bivalents	Total chiasmata	Terminal chiasmata	Total chiasmata per bivalent	Terminal chiasmata per bivalent
<i>A. baicalensis</i>					
Mid-diplotene	20	122	23	6.1	1.15
Late diplotene	58	186	38	3.2	0.66
Diakinesis	56	78	13	1.4	0.23
Metaphase	80	103	13	1.3	0.16
<i>A. sylvestris</i>					
Mid-diplotene	9	46	9	5.1	1.00
Late diplotene	9	28	6	3.1	0.66
Diakinesis	8	14	2	1.8	0.25
Metaphase	24	29	3	1.2	0.13
<i>A. sibirica</i>					
Mid-diplotene	—	—	—	—	—
Late diplotene	12	38	4	3.2	.33
Diakinesis	40	64	11	1.6	.27
Metaphase					
<i>A. borealis</i>					
Mid-diplotene					
Late diplotene	13	42	3	3.23	.23
Diakinesis					
Metaphase					
<i>A. sp.</i> (from Mr. T. Hay)					
Mid-diplotene	11	72	11	6.5	1.00
Late diplotene					
Diakinesis	32	58	9	1.8	0.28

Diagram I shows a graph plotted from these chiasma frequencies. (In plotting out the ordinates the stages are placed at equal distances as they are probably, approximately, equal in time. This however only affects the rate of terminalisation, and in no way invalidates the main conclusions to be drawn from the graph). For comparison the

frequency of total and terminal chiasmata per bivalent in *Campanula persicifolia* from data from Gairdner and Darlington (1932) has been plotted on the same graph. In *Campanula*, the terminalisation is normal and complete.

It will be noted that in *Anemone*, as the mean total number of chiasmata per bivalent falls there is a decrease in the number of terminal chiasmata per bivalent and at metaphase they both reach a minimum. In *Campanula* on the other hand a decrease in total number of chiasmata per bivalent is accompanied by a corresponding increase in the number of terminal chiasmata per bivalent until at metaphase the two coincide. There is a similar increase in the number of terminal chiasmata at metaphase in *Primula sinensis* (Darlington 1931).

Diagram I. Diagram showing the frequency of total chiasmata per bivalent in *Anemone baicalensis* and *Campanula persicifolia*. In *Anemone* the total number of chiasmata per bivalent decreases from diplotene to metaphase, and there is a corresponding decrease in the number of terminal chiasmata per bivalent. In *Campanula* where the terminalisation is of the normal type the number of terminal chiasmata per bivalent increases as the number of total chiasmata per bivalent decreases, until at metaphase the two curves coincide.

5. Significance of Observations

The behaviour of chromosomes at pachytene and the subsequent random formation of chiasmata indicates that the chromosome pairing in *Anemone* follows the usual course observed in other plants. The occurrence of a high percentage of terminal chiasmata shows that terminalisation takes place from very early stages, and the decrease in their number indicates that the connections at these terminal chiasmata are resolved at stages between diplotene and metaphase. The chiasmata move along the chromosomes away from the attachment constriction, but on reaching the ends, instead of remaining as terminal chiasmata they slip off, thus leaving the ends free. The occurrence of bivalents with interstitial chiasmata at metaphase indicates that the median chiasmata in the chromosomes do not move towards the ends as rapidly as do the more distal chiasmata. Such a condition is to be expected on the electrostatic theory of terminalisation put forward by Darlington and Dark (1932). According to this theory a single chiasma will not terminalise as rapidly as two chiasmata when one is on either side of the attachment constriction, (as observed in *Tulipa*, Darlington and Janaki-Ammal (1932)). The decrease in the number of interstitial chiasmata, at the same time as the decrease in the number of terminal chiasmata, indicates that the reduction in number is due to terminalisation and slipping off of the terminal chiasmata, rather than to a breaking of the interstitial chiasmata as might be assumed. This property of resolution of terminal chiasmata has not been observed before. In organisms examined so far, three types of chiasma behaviour have been observed. These types are as follows :

(1) Those in which there is no terminalisation, or where terminalisation takes place to a very slight degree. The resulting chiasmata are mostly interstitial at metaphase (*Hyacinthus*, *Uvularia*, *Vicia*, *Stenobothrus* etc).

(2) Those in which terminalisation is complete, and at metaphase the bivalents have terminal chiasmata (*Datura*, *Primula sinensis*, *Campanula*).

(3) Those with localised chiasmata, e.g. *Fritillaria Meleagris* (Newton and Darlington 1930) and *Mecostethus* (Janssens 1924). In *F. Meleagris* the chiasmata are always formed with a greater frequency towards the attachment constriction.

(There are, of course, forms whose behaviour is intermediate between Types 1 and 2).

If the terminalisation of chiasmata and subsequent slipping off at the ends were carried to an extreme condition it might possibly give rise to univalents at metaphase.

Darlington (1932) has postulated that the formation of terminal chiasmata and their retention at metaphase after terminalisation must be due to a special property of the ends of the chromosomes which he has described as "terminal affinity." The behaviour of *Anemone* is explained on this hypothesis by saying that the chromosomes have lost the property of terminal affinity. Such a lack of terminal affinity coupled with strong terminalisation might account for "asynapsis" at first metaphase in maize as found by Beadle (1931), and if such were the case crossing-over would occur in these asynaptic plants and could be explained on the theory of partial chiasmotypy.

6. Summary

(1) Most of the species of *Anemone* examined had a basic number of eight. *Anemone Hepatica*, a diploid species, had a basic number of seven ($2n = 14$). Somatic doubling was found in several root-tips of *A. Hepatica* and *A. vernalis*.

(2) Eight paired chromosomes can be distinguished at pachytene. The chiasma frequency at diplotene is 6.1 per bivalent. There is a fairly high percentage of terminal chiasmata per bivalent: a parallel reduction occurs in the total number of chiasmata per bivalent and the number of terminal chiasmata per bivalent between diplotene and metaphase. Most of the bivalents have a single interstitial chiasma at metaphase.

(3) The reduction in the number of total chiasmata per bivalent and terminal chiasmata per bivalent, indicates that terminalisation takes place and that the chiasmata are resolved on reaching the ends of the chromosomes. This can be interpreted as due to lack of terminal affinity.

(4) If this lack of terminal affinity were combined with strong terminalisation, univalent formation would result at metaphase although crossing-over had occurred.

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