Cytological observations on some species and hybrids of *Vaccinium*

ARNE ROUSI

Department of Horticulture, Agricultural Research Centre, Piikkiö

Summary. Meiosis was studied in *Vaccinium myrtillus* (n = 12), *V. vitis-idaea* (n = 12), *V. myrtillus* × *vitis-idaea* (n = 12), colchicine-induced tetraploid *V. myrtillus* (n = 24), *V. uliginosum* (n = 24), the cultivated blueberry varieties 'Rancocas' and 'Pemberton' (n = 24), and the hybrids *V. uliginosum* × 'Rancocas' and *V. uliginosum* × 'Pemberton' (n = 24). Pairing was regular in the diploid species. Some multivalent formations occurred in *V. uliginosum* and in the cultivated blueberry varieties and to a considerable extent in the autotetraploid *V. myrtillus*. The spontaneous diploid hybrid *V. myrtillus* × *vitis-idaea* displayed numerous meiotic irregularities, whereas the artificial hybrids between *V. uliginosum* and cultivated blueberry showed relatively regular meiosis. Pairing relationships of the various genomes are discussed. Breeding programs for the use of North-European species with American cultivated blueberry varieties are discussed in the light of the cytological observations.

Introduction

There are very few studies dealing with the cytology of the genus *Vaccinium*. Many chromosome counts have been made (e.g. LONGLEY 1927, HAGERUP 1933, NEWCOMER 1941, DARROW, CAMP, FISCHER and DERMEN 1944, LÖVE and LÖVE 1956, SORSA 1962), but meiotic phenomena at the different ploidy levels and in hybrids are practically unknown. Only the papers of LONGLEY (1927) and NEWCOMER (1941) contain very brief statements about meiosis. LONGLEY mentioned that the diploid, tetraploid and hexaploid species examined show a regular pairing in meiosis, whereas NEWCOMER found rings of four chromosomes in 'Rancocas', a cultivated blueberry variety of hybrid origin (containing genes of *V. australe*, *V. corymbosum* and *V. lamarckii*). He also observed secondary pairing and lagging chromosomes in some other cultivated varieties. Although there are a large number of natural and artificial interspecific hybrids in the genus (DARROW and CAMP 1945), very little is known about the chromosome pairing in these hybrids.

The present study was undertaken in connection with a breeding program with *Vaccinium*. The goal of this program is to produce a well adapted cultivated *Vaccinium* for Northern Europe by hybridizing American cultivated blueberry varieties with native North European species (ROUSI 1966). Since all North European species of *Vaccinium* (*Oxycoccus* excluded) are included in the breeding program, they are also included in this cytological investigation. These are the diploid species *V. myrtillus* L. and *V. vitis-idaea* L. and the tetraploid species *V. uliginosum* L. An autotetraploid *V. myrtillus* produced by colchicine, a spontaneous diploid hybrid *V. myrtillus* × *vitis-idaea*, and the artificial hybrid between *V. uliginosum* and cultivated blueberry were also studied, as well as the two cultivated blueberry varieties used for this cross, namely 'Rancocas' and 'Pemberton'. The purpose of the cytological investigation was to gain some knowledge about the pairing relationships of the chromosomes of different *Vaccinium* species, of the fertility of the hybrids and the autotetraploid mentioned above, as well as of the meiotic process in *Vaccinium* in general.

Material and methods

Flower buds of *V. myrtillus*, *V. vitis-idaea* and *V. uliginosum* were collected in 1963, 1964 and 1965 from natural populations in the vicinity of the Department of Horticulture, Agricultural Research Centre, Piikkiö (near Turku), southwestern Finland. The two former species were growing together in a pine forest, and *V. uliginosum* in a small bog on top of the hill Matinkallio.

Fig. 1.

Fig. 2.

Figs. 1–2. Two colchicine-induced tetraploid individuals of *Vaccinium myrtillus*.

The tetraploid *V. myrtillus* was obtained by colchicine treatment. Seeds were collected from the populations mentioned above in the fall of 1961. A portion was sown right away on filter paper in Petri dishes. After a few days, when the seeds started to germinate, they were immersed in a colchicine solution (0.25 or 0.5 per cent) for 3, 6 or 9 hours. Other seeds were sown in the spring of 1962 in flats and the growing points of the young seedlings were treated with 0.25 or 0.125% colchicine solution. These treatments lasted 24 hours each but were repeated up to 8 times at 48 hours intervals. In the

fall of 1962 around 100 seedlings were planted in a cold frame. The first of them flowered in the spring of 1964. Screening for tetraploid plants was carried out by measurements of the stomata and pollen tetrads, determinations of pollen stainability, as well as squash preparations of either very young leaves or anthers. Two individuals were found which were tetraploid at least in most parts of their tissues. They are called here A and B. A (Fig. 1) had been immersed in 0.5 per cent colchicine solution for 6 hours when germinating in 1961, B (Fig. 2) had received 7 treatments of 0.25 per cent solution in 1962. The individual B was small and dark and had characteristically rounded, thick leaves. This individual never flowered and it died in the spring of 1965. The individual A was vigorous and flowered both in 1964 and 1965, so that it was possible to study its meiosis and ascertain that the PMCs contained a tetraploid chromosome number. It had somewhat larger and definitely broader leaves than the diploids (Fig. 3).

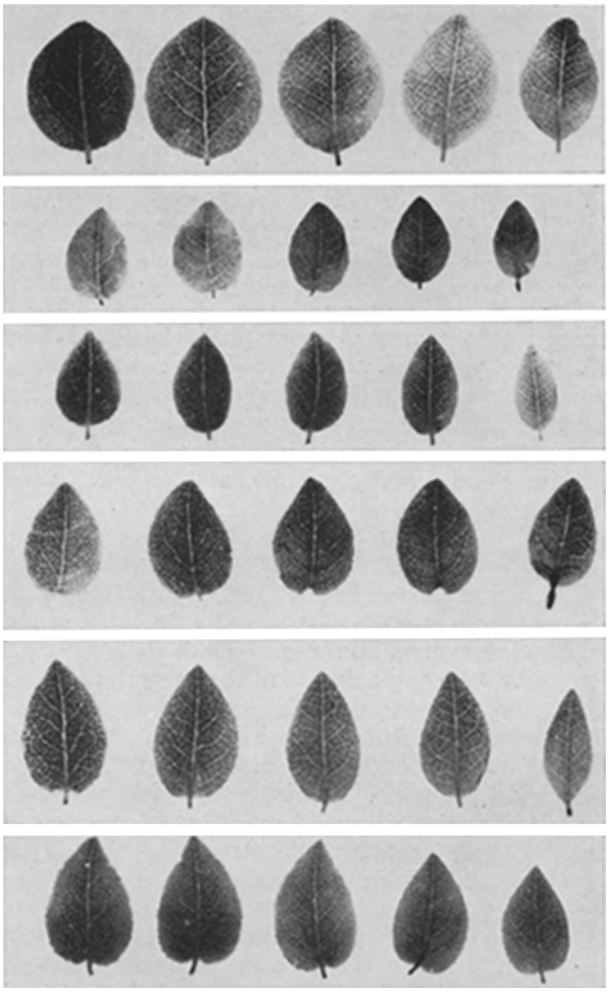

Fig. 3. Series of five apical leaves of a tetraploid (uppermost row) and five diploid individuals of *V. myrtillus*. The silhouettes were prepared by illuminating photographic paper through the leaves. Through the paper negative so obtained another sheet of photographic paper was illuminated to obtain the positive. × 0.8.

Living plants of *V. myrtillus* × *vitis-idaea* were obtained from the Royal Botanic Gardens, Edinburgh, in 1962. This spontaneous hybrid is also called *V. intermedium* Ruthe (cf. RITCHIE 1955a, 1955b). Flower buds were fixed in 1964 and 1965 from individuals growing in the experimental field.

The F_1 hybrids between *V. uliginosum* and the cultivated blueberry varieties 'Rancocas' and 'Pemberton' were obtained from crosses made in 1961 (ROUSI 1963). The F_1 progenies, which were intermediate for most characters (Fig. 4), flowered for the first time in 1964. Flower buds were fixed from several individuals of both progenies in 1964 and 1965. For control, flower buds were also fixed from 'Rancocas' and 'Pemberton' in the same years.

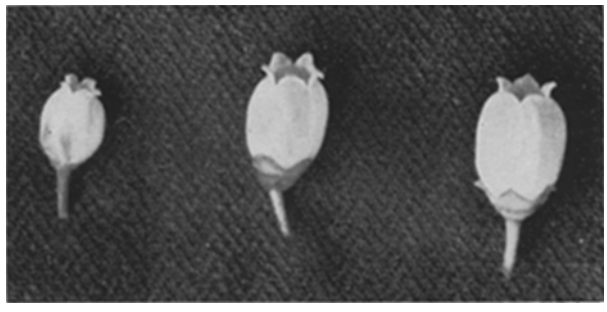

Fig. 4. Flowers of *Vaccinium uliginosum* (left), 'Rancocas' (right) and their F_1 hybrid (middle).

Both squash and paraffin methods were used for studying meiosis in all the species and hybrids. The buds used for the squash preparations were fixed in acetic alcohol. After fixation they were placed on a watch-glass in a mixture of propionic orcein or acetic orcein (9 parts) and 1 N HCl (1 part), and warmed above a flame for a few seconds. The anthers were then taken from the bud and squashed in pure propionic or acetic orcein. A phase contrast microscope WILD M 20 was used. If the fixation in acetic alcohol was omitted and the buds taken directly into the mixture of HCl and acetic orcein, the chromosomes appeared less distinct. If the Feulgen method of staining was used, the cytoplasm appeared granular with phase contrast optics. The staining of the chromosomes was so weak, on the other hand, that the use of phase contrast was necessary.

When the paraffin method was used, the flower buds were fixed in Craf, and embedded and sectioned in the normal way. The slides were stained first with the Feulgen method and then immediately with crystal violet. This double staining gave a much better contrast than either the Feulgen method or crystal violet alone. The same effect could previously be seen in *Potentilla anserina* L. (ROUSI 1965) and it seems that this double staining may have a wide application to plant material with small, poorly staining chromosomes. Because of the good staining in the sectioned preparations, they were studied with an ordinary microscope (WILD M20 Fluotar). All illustrations are made from squash preparations, however.

Observations on the meiotic prophase

Particularly in *V. uliginosum* and its hybrid with American blueberry varieties, some observations were made on the prophase stages of meiosis. The beginning of the meiotic prophase was characterized by a bouquet formation (Fig. 5), which later began to spread out (Fig. 6) to form a typical pachytene stage. The nucleolus and next to it a heteropycnotic knob, presumably a nucleolar organizer, were clearly visible at this stage (Fig. 6). The diplotene —

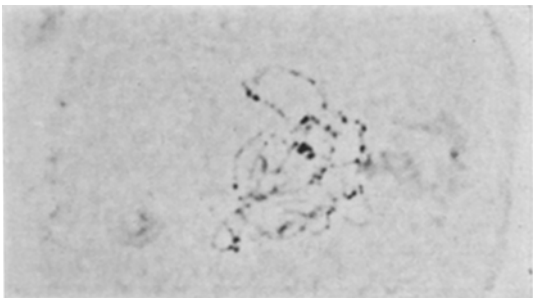

Fig. 5.

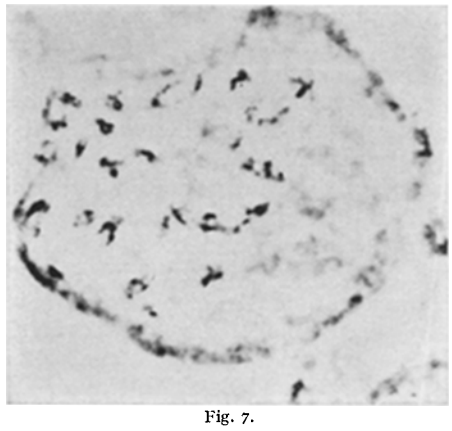

Fig. 7.

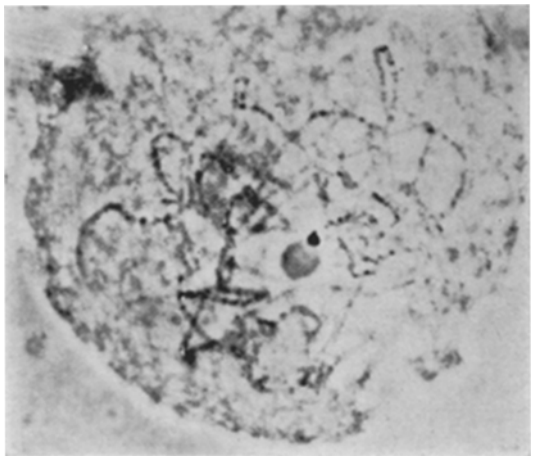

Fig. 6.

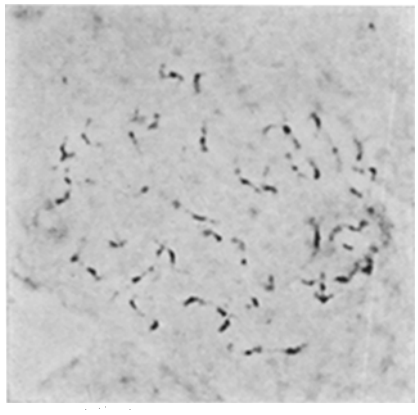

Fig. 8.

Figs. 5–8. Photomicrographs of meiotic and mitotic prophase stages. Fig. 5. A zygotene bouquet in *V. uliginosum*. Fig. 6. Pachytene in *V. uliginosum* × 'Rancocas'. Fig. 7. Early diakinesis in *V. uliginosum* × 'Rancocas'. Fig. 8. A mitotic prophase in *V. uliginosum*. ×1500.

diakinesis stages (Fig. 7) showed bivalents which could be counted in some cases. Heteropycnotic segments of chromosomes were visible at this stage (Fig. 7), a phenomenon which was even clearer at some of the mitotic prophases (Fig. 8), where every chromosome had a distinct heteropycnotic segment, mostly in the central part.

Observations on diakinesis and later stages of meiosis

The diploid species Vaccinium myrtillus and V. vitis-idaea

The meiosis in the PMCs of *V. myrtillus* took place at the end of April and that of *V. vitis-idaea* around the middle of May at Piikkiö. In 1964, when the spring was late, meiosis occurred about a week later in both.

As far as can be judged from the material available, the meiosis of these two diploid species shows no exceptional features At metaphase I both *V. myrtillus* (Fig. 9) and *V. vitis-idaea* (Fig. 11) were characterized by very regular pairing, 12 bivalents being the rule in all the cells analyzed. The segregations at anaphase I (Fig. 10) and at the second meiotic division were equally normal, and regular tetrads were formed.

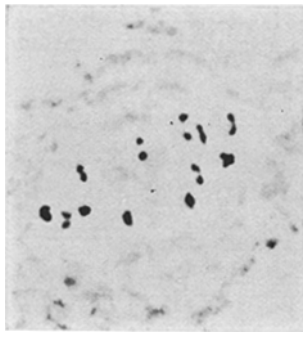

Fig. 9.

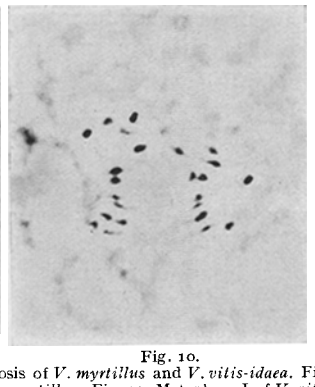

Fig. 10.

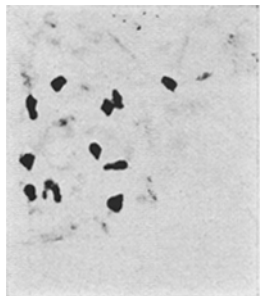

Fig. 11.

Figs. 9–11. Photomicrographs of meiosis of *V. myrtillus* and *V. vitis-idaea*. Fig. 9. Metaphase I in *V. myrtillus*. Fig. 10. Anaphase I of *V. myrtillus*. Fig. 11. Metaphase I of *V. vitis-idaea*. ×1500.

V. myrtillus × vitis-idaea

The meiosis in the PMCs of this hybrid took place during the first half of May at Piikkiö. There was repeated flowering, however, towards the end of the summer. As compared with the parental species, a considerable number of disturbances appeared. The degree of chromosome pairing was very variable. Figs. 12 and 13 show two diakinesis nuclei where pairing is complete, 12 bivalents being visible in each. At this stage it could be seen that a considerable proportion of the bivalents were ring-shaped. At metaphase I univalents were very frequent, often lying outside the metaphase plate. Sometimes as many as 12 univalents could be counted (Fig. 14). No multivalents were seen. The segregation at anaphase I was sometimes regular (Fig. 15), but very often laggards were observed (Fig. 16). Of 100 PMCs which were counted at anaphase I, 46 showed no laggards, 21 showed one laggard, 15 two laggards, 13 three laggards and 5 four or more laggards. The

second meiotic division also displayed many irregularities, including laggards and unequal distributions of chromosomes. For example, Fig. 17 shows an anaphase II where two of the chromosome groups contained 14, and two 10 chromosomes.

As a result of the meiosis in the PMCs, regular-looking tetrads were mostly formed. Apparently the numerous laggards which could still be seen at anaphase II either fused with the main nuclei or were eliminated before the tetrad stage. As a result of the disturbances, a large proportion of the nuclei obviously contained irregular chromosome constitutions.

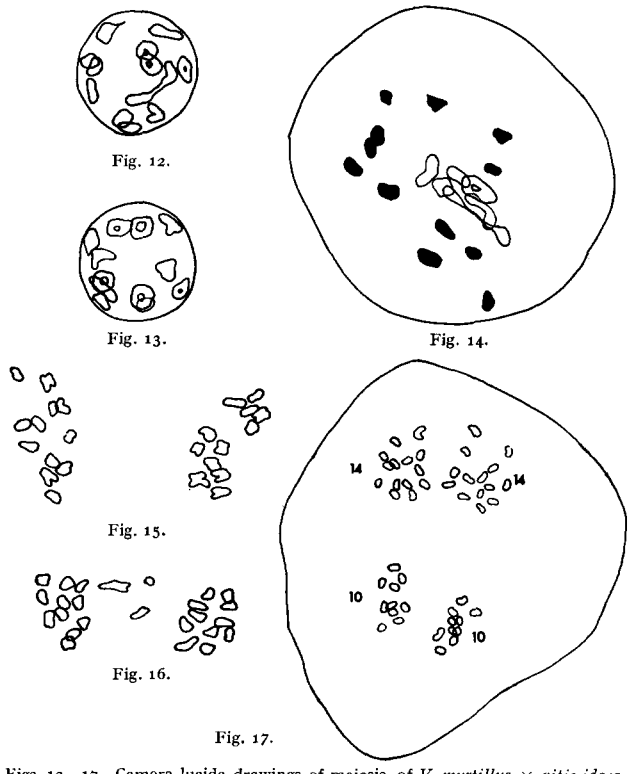

Fig. 12. Fig. 13. Fig. 14. Fig. 15. Fig. 16. Fig. 17.

Figs. 12—17. Camera lucida drawings of meiosis of *V. myrtillus* × *vitis-idaea*. Fig. 12. Diakinesis with 12 bivalents. Fig. 13. Diakinesis with 12 bivalents. Fig. 14. Metaphase I with 12 univalents. Fig. 15. Regular segregation (12 + 12) at anaphase I. Fig. 16. Anaphase I with laggards. Fig. 17. Anaphase II with an irregular segregation of chromosomes. × 2000.

To obtain an idea of the pollen fertility, 10 pollen samples, taken from different flowers at different times, were analyzed in regard to stainability with cotton blue. In each sample, 100 randomly selected pollen tetrads were studied (as in the whole family of *Ericaceae*, the pollen grains remain in tetrads). As is shown in Table 1, the proportion of stained pollen grains was 45.3 in the hybrid and 92.0 in *V. myrtillus* (2*x*), which was analyzed in the same way. This difference reflects corresponding differences in pollen fertility. RITCHIE (1955a) made germination experiments with pollen of the hybrid and both parental species. The mean germination percentages were 65.9 in *V. myrtillus*, 67.2 in *V. vitis-idaea* and 4.4 in the hybrid, indicating a low pollen fertility of the hybrid.

The vigorously growing hybrid flowered abundantly, the main periods of flowering occurring in June and August—September. Extremely few fruits were formed, and only at the end of the growing season. Four berries contained numerous aborted ovules and, in all, 10 fully developed seeds. When these were stratified and sown, normal-looking F_2 plants were obtained. The hybrid clones studied by RITCHIE (1955a) had a somewhat higher berry production, 9 per cent of the number of flowers (the corresponding frequencies being 65 in *V. myrtillus* and 59 in *V. vitis-idaea*). However, the mean number of seeds per berry in RITCHIE's hybrids was only 2 and thus very close to the present observations.

It seems that although the chromosomes of *V. myrtillus* and *V. vitis-idaea* are capable of pairing in some cases, there are impediments to regular pairing. Since pairing is sometimes complete and no inversion bridges or multivalents were observed, it does not seem probable that any large chromosomal rearrangements differentiate the chromosome sets of the two species. It seems rather that there may be a number of small structural differences which cause irregularities in pairing and even in complete pairing lead to the formation of pollen grains and embryo sacs with duplications and deficiencies (cryptic structural hybridity of STEBBINS 1950). The hybrid as well as F_2 individuals have repeatedly been treated with colchicine and the treatments will be continued in order to obtain tetraploid shoots. It remains to be seen whether allotetraploidy will lead to preferential pairing and improved fertility.

Tetraploid V. myrtillus

The meiosis of this colchicine-induced autotetraploid was studied in 1965. Quadrivalents and trivalents were rather frequent at metaphase I (Fig. 18) and univalents were also found, apparently as a result of a combination trivalent + univalent. The univalents were often situated outside the metaphase plate. Anaphases I and II mostly looked regular, although laggards were rather common. Of 50 anaphases I, counted at random, 38 looked normal, 10 contained one laggard, 1 contained two and 1 con-

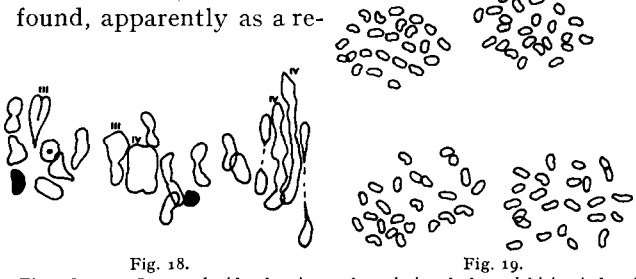

Fig. 18. Fig. 19.

Figs. 18—19. Camera lucida drawings of meiosis of the colchicine-induced tetraploid *V. myrtillus*. Fig. 18. Metaphase I with 3 quadrivalents, 2 trivalents, 14 bivalents and 2 univalents. Fig. 19. Regular segregation of chromosomes at anaphase II. × 2000.

Table 1. *The stainability of pollen tetrads with cotton blue.*

	The percentages of tetrads with 4 to 0 stained pollen grains					The percentage of stained pollen grains
	4	3	2	1	0	
V. myrtillus (2*x*)	86.9	5.8	0.7	1.5	5.1	92.0
V. myrtillus (4*x*)	60.2	17.6	3.6	9.0	9.6	77.5
V. myrtillus × *vitis-idaea*	18.9	20.9	7.5	27.7	25.0	45.3
V. uliginosum	75.0	14.0	2.0	3.0	6.0	87.3
'Rancocas'	78.0	16.0	3.0	0.0	3.0	91.5
V. uliginosum × ('Rancocas', 'Pemberton')	76.1	10.7	1.3	3.6	8.3	85.7

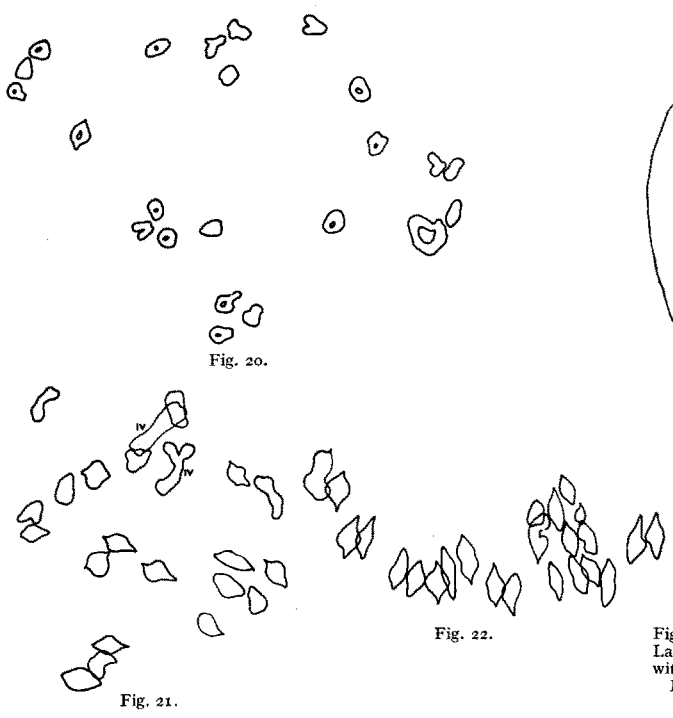

Fig. 20.

Fig. 22.

Fig. 21.

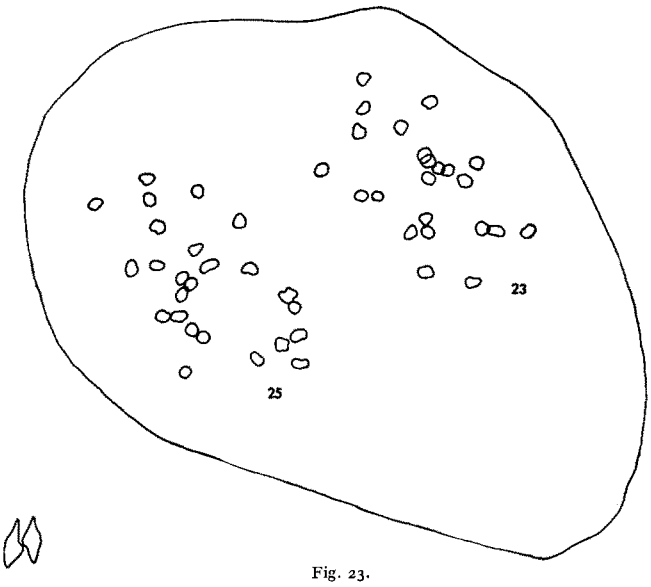

Fig. 23.

Figs. 20–23. Camera lucida drawings of meiosis of *V. uliginosum*. Fig. 20. Late diakinesis with 1 ring quadrivalent and 22 bivalents. Fig. 21. Metaphase I with 2 quadrivalents and 20 bivalents. Fig. 22. Metaphase I with 24 bivalents. Fig. 23. Anaphase I with an irregular segregation of chromosomes. × 2000.

tained three laggards. Unequal distributions were observed, but a perfectly equal distribution (24 + 24 + 24 + 24) could also be counted at anaphase II (Fig. 19).

Five pollen samples, collected at different times and from different flowers, were stained with cotton blue and 100 pollen tetrads of each were studied. The results of these determinations are given in Table 1. The percentage of stained pollen grains, 77.5, was considerably lower than in diploid *V. myrtillus*. The reduced fertility was also shown by the fact that no berries were formed in the tetraploid *V. myrtillus*. At the same time, adjacent diploid individuals of the same age were fruiting abundantly.

V. uliginosum

HAGERUP (1933) showed that *V. uliginosum* L. s. lat. consists of a diploid (2 n = 24) race, found by him in Greenland, and a more widespread tetraploid (2 n = 48) race. The diploid race is morphologically distinct and usually known as *V. uliginosum* subsp. *microphyllum* Lge., *V. microphyllum* (Lge) Hagerup or *V. gaultherioides* Bigel. LÖVE and LÖVE (1965) consider it to be a distinct species and point out that *V. gaultherioides* Bigel. is its correct name. They were able to show that the diploid also occurs in North America and in mountains of Eurasia south of the area of the tetraploid. HARA (1953) reported a hexaploid count (2 n = 72) from Japan. It seems that the tetraploid race is the most widespread, however. The strain used for the present study was tetraploid, as are probably all Finnish strains of *V. uliginosum*.

Meiosis in the PMCs occurred at Piikkiö during the first half of May. The meiotic configurations could be studied at diakinesis and metaphase I. The majority of chromosomes occurred as bivalents, but multivalents were also common (Figs. 20–22). These could mostly be identified as quadrivalents and were often ring-shaped. Especially at diakinesis it was apparent that the majority of bivalents were ring-shaped.

The anaphases were mostly regular-looking. Of 50 anaphases I, counted randomly, 46 looked normal and 4 contained at least one laggard. There was some evidence, however, that the segregation of chromosomes was abnormal even though no laggards were present. This probably resulted from unequal division of multivalents. Thus Fig. 23 shows an anaphase I with 23 chromosomes in one group and 25 in the other. Similarly, the stainability of the pollen indicated somewhat reduced fertility. Of 100 pollen tetrads, 87.3 per cent of the pollen grains were stained (Table 1).

Highbush blueberry (mostly V. corymbosum and V. australe)

Meiosis was studied in the highbush blueberry varieties 'Rancocas' and 'Pemberton', the two varieties used in hybridization with *V. uliginosum* (ROUSI 1963). These varieties themselves have evolved from interspecific crosses, as is shown by Fig. 24.

Meiosis in the PMCs of 'Rancocas' and 'Pemberton' at Piikkiö stretched over a relatively long period, but usually started near the middle of May. Meiosis was rather regular, except for the fact that multivalents were very frequent at metaphase I. One or

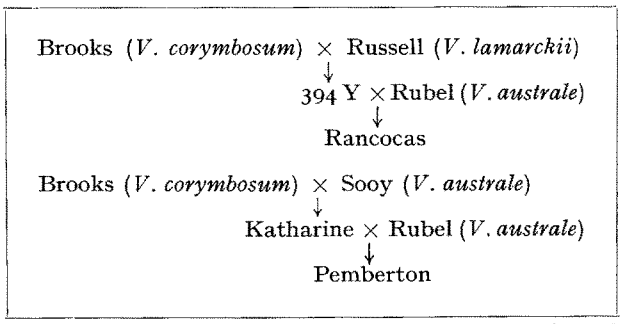

Fig. 24. Ancestry of the blueberry varieties 'Rancocas' and 'Pemberton', according to COVILLE (1937) and DARROW (1960).

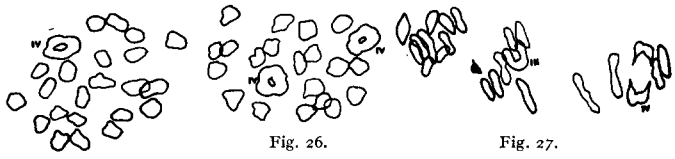

Fig. 25. Fig. 26. Fig. 27.

Figs. 25–27. Camera lucida drawings of meiosis of cultivated highbush-blueberry. Fig. 25. Metaphase I in 'Rancocas' with 1 quadrivalent and 22 bivalents. Fig. 26. Metaphase I in 'Rancocas' with 2 quadrivalents and 20 bivalents. Fig. 27. Metaphase I in 'Pemberton' with 1 quadrivalent, 1 trivalent, 20 bivalents and 1 univalent. ×2000.

two ring quadrivalents were commonly observed (Figs. 25–27). Combinations trivalent + univalent could also be seen at metaphase I (Fig. 27). These obviously result from falling apart of pachytene quadrivalents with an insufficient number of chiasmata. NEWCOMER (1941) also observed ring quadrivalents at the meiotic metaphase I of 'Rancocas'. In the other varieties studied by him he did not observe multivalents, but he mentioned secondary pairing and lagging in a few instances. 'Pemberton' was not among the varieties studied by him. Later studies will perhaps reveal whether multivalent formation is restricted to 'Rancocas', 'Pemberton' and perhaps some other varieties or whether it is universal among the highbush blueberry varieties.

The later stages of meiosis were mostly normal in both varieties. The segregation of the multivalents at anaphase I obviously does not involve any great difficulties and other disturbances are practically lacking.

The stainability with cotton blue was determined in a sample of 100 pollen tetrads of 'Rancocas' (Table 1).

V. uliginosum × *cultivated blueberry*

Meiosis in the PMCs of the hybrids *V. uliginosum* × 'Rancocas' and *V. uliginosum* × 'Pemberton' occurred at about the same time as that of *V. uliginosum*, i.e. during the first half of May. Pairing was surprisingly regular, 24 bivalents being the commonest situation at metaphase I (Figs. 28, 29). Multivalents, such as ring quadrivalents (Fig. 30) or chain trivalents (Figs. 31, 32), were also found, as well as univalents (Figs. 33, 34). In the hybrid *V. uliginosum* × 'Rancocas' 25 metaphase I cells were analyzed completely. These represented two different F_1 individuals. Altogether, these cells contained 5 groups of four, 2 groups of three, 580 bivalents and 14 univalents. This means that one cell contained an average of 23.2 bivalents, and 96.7 per cent of the chromosomes paired as bivalents. The situation was very similar in *V. uliginosum* × 'Pemberton' where 12 metaphase I cells, also representing two F_1 individuals, were analyzed. They contained 1 group of four, 2 groups of three, 280 bivalents and 6 univalents. One cell thus contained an average of 23.3 bivalents, and 97.2 per cent of the chromosomes paired as bivalents. Disturbances of pairing were thus comparatively rare, especially when one takes into account the fact that the parental species also had a certain number of multivalents and univalents at meiosis.

The segregation of chromosomes at anaphase I was in most cases a normal 24 + 24 (Fig. 35). The second meiotic division was also mostly normal, leading to the formation of normal tetrads. The stainability of pollen tetrads with cotton blue was checked in 7 samples of 100 tetrads (Table 1). The percentage of pollen grains which stained was 85.7 as an average, varying from 74.3 to 94.8 in different individuals. This percentage varied, in other words, from somewhat lower to slightly higher as compared with the corresponding percentages observed in the parental species (87.3 in *V. uliginosum* and 91.5 in 'Rancocas').

Several back-crosses were made in 1965 between various F_1 individuals on the one hand and highbush blueberry varieties 'Rancocas', 'Pemberton' and 'Bluecrop' on the other hand. From the crosses where the F_1 was used as the mother parent, 10 berries were obtained, containing as an average 4.6 fully developed seeds. This may serve as a rough measure of the seed fertility of the hybrid. When one of the three blueberry varieties was used as mother parent, 199 seeds were obtained from 69 berries, which gives an average of 2.9 fully developed seeds per berry. A good

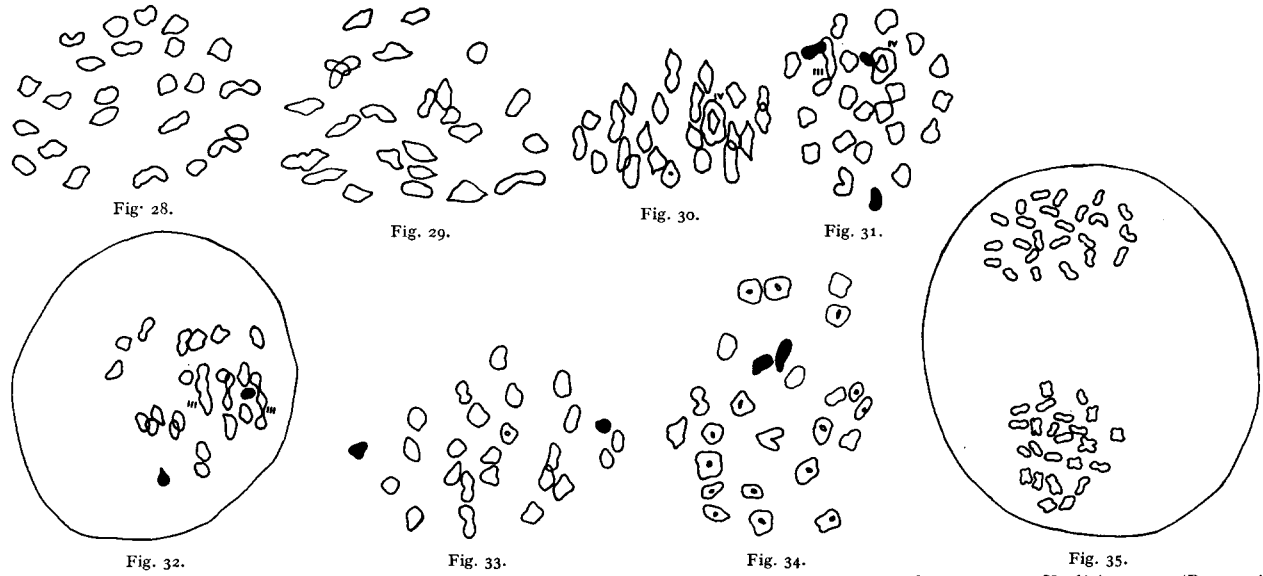

Fig. 28. Fig. 29. Fig. 30. Fig. 31.

Fig. 32. Fig. 33. Fig. 34. Fig. 35.

Figs. 28–35. Camera lucida drawings of meiosis of *V. uliginosum* × cultivated blueberry. Figs. 28, 30, 31, 33, and 35 represent *V. uliginosum* × 'Rancocas', Figs. 29, 32 and 34 *V. uliginosum* × 'Pemberton'. Fig. 28. Metaphase I with 24 bivalents. Fig. 29. Metaphase I with 24 bivalents. Fig. 30. Metaphase I with 1 quadrivalent and 22 bivalents. Fig. 31. Metaphase I with 1 quadrivalent, 1 trivalent, 19 bivalents and 3 univalents. Fig. 32. Metaphase I with 2 trivalents, 20 bivalents and 2 univalents. Fig. 33. Metaphase I with 23 bivalents and 2 univalents. Fig. 34. Metaphase I with 23 bivalents and 2 univalents. Fig. 35. Regular segregation at anaphase I. ×2000.

germination of the back-cross seeds took place in 1966.

Discussion

The most regular meiosis within the material studied was found in the two diploid species *Vaccinium myrtillus* and *V. vitis-idaea*. The autotetraploid *V. myrtillus* had a number of irregularities, particularly multivalents. It is noteworthy that the meiosis of the tetraploid *V. uliginosum* also displayed a certain number of irregularities, including multivalents. It seems probable that this species is an autotetraploid of *V. gaultherioides* Bigel. (= *V. uliginosum* L. subsp. *microphyllum* Lge.). The fact that it has retained meiotic features of typical autopolyploids suggests that its origin is relatively recent, possibly connected with the Pleistocene glaciation.

The tetraploid cultivated blueberry varieties 'Rancocas' and 'Pemberton' also displayed multivalent formation. They are derived from interspecific crosses used in the breeding work of highbush blueberry (Fig. 24). The whole subgenus *Cyanococcus* in Eastern North America forms a large polyploid complex in which both auto- and allopolyploidy have played significant roles and which has undergone considerable evolution during and after the Pleistocene glaciation (CAMP 1942, 1945). It is to be expected, therefore, that multivalents may occur in the meiosis of tetraploid representatives of the group.

The diploid hybrid *V. myrtillus* × *vitis-idaea* displayed a number of meiotic irregularities, while the tetraploid hybrids between *V. uliginosum* and cultivated blueberries had a remarkably regular meiosis. The chromosome sets of *V. myrtillus* and *V. vitis-idaea* have obviously developed differences which prevent regular pairing in the hybrid and probably also give rise to a proportion of genetically anomalous products of meiosis, even when complete pairing occurs (cryptic structural hybridity of STEBBINS, 1950).

The nearly perfect pairing and fertility of *V. uliginosum* × 'Rancocas' and *V. uliginosum* × 'Pemberton' suggests that the chromosome set of the former species is to a large extent homologous with that of the representatives of subg. *Cyanococcus*. An alternative explanation would be that autosyndesis occurs in the hybrid, *uliginosum* chromosomes pairing with one another and *Cyanococcus* chromosomes doing the same. This would imply that the *uliginosum* chromosomes and *Cyanococcus* chromosomes are not necessarily able to pair, except when multivalent formation occurs. It would mean, however, that all four genomes of *V. uliginosum* are able to pair, as well as all four genomes of 'Rancocas' and 'Pemberton', on the other hand. This does not seem likely, at least not in 'Rancocas' and 'Pemberton', which are of rather complex hybrid origin. Both in these and in *V. uliginosum* the incidence of multivalents is so low that almost complete autosyndesis in the hybrid does not seem very likely. There is no definite proof against it, however.

For breeding purposes, the hybrids *V. uliginosum* × 'Rancocas' and *V. uliginosum* × 'Pemberton' were backcrossed in 1965 to highbush blueberry varieties, the ultimate goal being a plant which would combine the climatic adaptation of *V. uliginosum* with the good fruit quality and productivity of the cultivated blueberry (cf. ROUSI 1966). The result of this backcross will be different depending upon the way of pairing in F_1. If the *uliginosum* genomes are denoted as U and the *Cyanococcus* genomes as C, the F_1 hybrid has the constitution UUCC. If allosyndesis takes place, the gametes of F_1 will contain all possible combinations of the U and C genomes and the backcross progeny will vary from *Cyanococcus* types to F_1-like types (UUCC), although the occurrence of types quite as extreme is very unlikely. If autosyndesis takes place, all gametes of the F_1 will be of the type UC and the backcross progeny entirely of the type UCCC and probably rather uniform. From the breeding point of view, the former situation would be more promising, since the first backcross progeny would provide rich material for selection.

The breeding plans also include the production of a tetraploid from *V. myrtillus* × *vitis-idaea* and the use of such an allotetraploid in breeding work on the tetraploid level with American cultivated varieties. The autotetraploid *V. myrtillus* would be used similarly for breeding at the tetraploid level (ROUSI 1966). The present study indicates that such uses may be possible. The autotetraploid *V. myrtillus* seems to be fertile enough for breeding work, and there is reason to assume that a tetraploid produced from *V. myrtillus* × *vitis-idaea* would have an improved fertility. Since some pairing takes place on diploid level, the tetraploid should probably be classified as a segmental allopolyploid.

Zusammenfassung

Die diploiden (n = 12) Arten *Vaccinium myrtillus*, *V. vitis-idaea*, *V. myrtillus* × *vitis-idaea* und die tetraploiden (n = 24) *V. myrtillus* (Colchicin-induziert) und *V. uliginosum* sowie die kultivierten amerikanischen Blaubeersorten 'Rancocas' und 'Pemberton' (n = 24) und die Hybriden *V. uliginosum* × 'Rancocas' und *V. uliginosum* × 'Pemberton' wurden cytologisch untersucht. In den diploiden Arten war die meiotische Chromosomenpaarung regelmäßig. Multivalente traten in geringerem Umfange bei *V. uliginosum* und kultivierten Blaubeersorten, viel stärker aber in der autotetraploiden *V. myrtillus* auf. Die spontane diploide Hybride *V. myrtillus* × *vitis-idaea* zeigte zahlreiche meiotische Störungen, die künstlichen Hybriden zwischen *V. uliginosum* und der kultivierten amerikanischen Blaubeere hatten demgegenüber eine relativ regelmäßige Meiose. Die Paarungsverhältnisse der verschiedenen Genome werden diskutiert. Auf Grund der cytologischen Beobachtungen wird ein Züchtungsprogramm für nordeuropäische *Vaccinium*-Arten zusammen mit amerikanischen Kultursorten erörtert.

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Hat J. G. MENDEL bei seinen Versuchen „zu genau" gearbeitet? – Der χ^2-Test und seine Bedeutung für die Beurteilung genetischer Spaltungsverhältnisse*

F. WEILING

Institut für landwirtschaftliche Botanik der Universität Bonn

Has J. G. MENDEL been "too accurate" in his experiments? The χ^2 test and its significance to the evaluation of genetic segregation

Summary. In a statistical analysis of GREGOR MENDEL's experiments R. A. FISHER (1936) expresses the opinion that from a statistical point of view MENDEL's experimental results are too exact. Assuming that MENDEL recognized the regularities of segregation already from his first seed counts in 1858, Fisher believes that further experimentation by MENDEL was only of demonstrative value. Several authors, f.i. C. Zirkle (1964) share this opinion.

However, FISHER and the other authors have overlooked that in judging F_3 analyses of MENDEL's experiments, when not seed characteristics were tested but plant traits that made raising an F_3 necessary, the number of progeny available for classification could hardly be equal to the 10 seeds planted or presumably planted, since one has to count on losses through poor germination, birds, or other causes. We show that with an assumed average number of 8 plants in these progenies the probability of agreement with expectation in all of MENDEL's experiments is numerically equal to the probability calculated from experiments with peas by CORRENS (1900), TSCHERMAK (1900), BATESON and KILLBY (1905), as well as by DARBISHIRE (1908, 1909), the latter totaled.

The too great seeming exactness of the experiments with peas could be explained in the following manner: The distribution of genetic segregation data, obviously different for each plant or animal species, is not binomial but "semirandom", therefore the calculated χ^2 value will be too small.

We try to estimate the factor c by which the χ^2 value in experiments on peas is too small, and to determine the consequences of this fact to the validity of the χ^2 test. For comparison we point out results from similar investigations on several plant and animal species, again using data from the literature.

Einleitung

Im Jahre 1936 veröffentlichte R. A. FISHER einen Aufsatz unter dem Titel: „Ist MENDELs Arbeit eigentlich wiederentdeckt worden?" In ihr geht er dem statistischen Gehalt der MENDELschen Arbeit nach.

FISHER stellt fest, daß nicht nur MENDELs Zeitgenossen ihn nicht verstanden, da sie in seiner Arbeit nur eine Wiederholung bereits allgemein bekannter Kreuzungsergebnisse sahen, sondern daß auch die Wiederentdecker ihn nur insoweit begriffen, als ihnen der Stand der Forschung die Voraussetzungen für ein Verständnis gab.

A. Die statistischen Einwände gegen MENDELs Arbeit

Auch MENDEL gegenüber meldet FISHER Vorbehalte an. Er prüft die Frage, ob die Versuche in der Weise durchgeführt werden konnten, wie MENDEL sie geschildert hat, und kommt zur Feststellung, daß MENDELs Ausführungen zur Versuchsdurchführung zwar wörtlich genommen werden müssen. Indessen ergibt die mit Hilfe des χ^2-Testes durchgeführte Prüfung, daß der Genauigkeitsgrad der Versuche zu hoch ist, andererseits MENDEL in zwei Fällen, nämlich bei der Beurteilung der F_3-Spaltungen der mono- und trihybriden Kreuzungen von falschen Erwartungswerten ausgeht.

Die übergroße statistische Genauigkeit aller Versuche sowie der Umstand, daß die Übereinstimmung der beobachteten mit den auf Grund falscher Erwartung sich ergebenden Vergleichswerten gleichfalls eine recht gute ist, läßt ihn die Frage stellen, ob die angegebenen Zahlen echt seien. Zwar zweifelt FISHER die Ehrlichkeit MENDELs nicht an; aber er meint, MENDEL habe vielleicht eine Hilfe gehabt und diese habe möglicherweise in Kenntnis der erwarteten Werte die beobachteten Daten leicht frisiert. Da MENDEL ein guter Pädagoge gewesen sei, andererseits der Gedanke nahe liege, daß MENDEL bereits zu Beginn seiner Arbeit (etwa nach Auszählung der im Jahre 1858 im Verhältnis 3:1 spaltenden Samenmerkmale) die dem Vererbungsgeschehen zugrunde liegende Gesetzmäßigkeit erkannt habe, sei anzunehmen, daß

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