Fertility and Chromosome Behavior of a Derived Decaploid of *Vaccinium*[1]

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Abstract. Chromosome pairing in a derived decaploid, 2n=10×=120, (from a colchicine-treated, sterile pentaploid hybrid of *Vaccinium ashei* Reade × *V. corymbosum* L.) was chiefly bivalent. Multivalent associations were present in 5 of 16 cells scored at metaphase I, but only 23 chromosomes of the 1,920 observed were involved in multiple pairing. Most of the cells at anaphase I (33 of 35 observed) contained lagging chromosomes which appeared to be non-disjoined bivalents rather than univalents. The decaploid was selfed and crossed with hexaploid, tetraploid, and diploid species. The mean number of seedlings produced per flower pollinated for each ploidy level was low, ranging from 0.04 to 0.09. The mean seedling number produced per self-pollinated flower was considerably higher, 0.92. The percent of pollen which stained in propionocarmine was 8% for the pentaploid parent, 62% for the derived decaploid and 99% for plants of *V. ashei* and *V. corymbosum* used as standards for comparison.

An induced decaploid blueberry has a low level of fertility in crosses with various species of *Vaccinium*. This study was undertaken to determine whether the sterility is associated with meiotic irregularities.

Moore, Scott and Dermen (5) reported the doubling of the chromosome number by colchicine treatment of shoot buds of a sterile pentaploid selection, 2n=5×=60. The pentaploid was a hybrid from a cross of tetraploid highbush blueberry, *Vaccinium corymbosum,* cv. Bluecrop, and hexaploid rabbiteye blueberry, *V. ashei* cv. Myers. The decaploid is of interest because of the possibility it offers of combining the characteristics of both species in a fertile hybrid for further breeding, and because few such high level polyploids are available for meiotic study.

The originators of the decaploid attempted no meiotic study, but reported that pollen of the pentaploid, 'T-17,' was mostly irregular in size and shape and that of the decaploid, 'US 40', was more uniform. This implies more regular meiosis and at least some restored fertility in the decaploid. It would be expected that after doubling, the 2 chromosome sets from *V. corymbosum* would pair as would the 2 chromosome sets of *V. ashei,* giving a normal meiosis. However, we have failed to obtain populations large enough for meaningful selection either by selfing 'US 40', or in crosses with heteroploid genotypes. There are no other decaploids of *Vaccinium;* therefore, the fertility of 'US 40' was determined in this study from crosses with hexaploid, tetraploid, and diploid species and from self-pollinations.

Materials and Methods

All crosses were made in the greenhouse with potted plants in late winter after sufficient chilling of all genotypes. Flowers to be used as female were emasculated just before opening and pollinated 2 successive days with freshly collected pollen. Seed was extracted from the fruit, dried and refrigerated in vials until the following winter. They were sown in the greenhouse on the surface of flats of milled sphagnum moss. This is the same procedure used with seed from ordinary breeding work. The number of seedlings produced per pollinated flower in each cross was used as the criterion of fertility rather than number of seeds. Counting small blueberry seed is laborious and it is often difficult to visibly distinguish fully developed seed from abortive seed.

Genotypes used as male parents in crosses with 'US 40' as female were grouped according to 4 ploidy levels, diploid, tetraploid, hexaploid and decaploid. The number of seedlings obtained was used as the number of functional eggs for each cross. The number of non-functional eggs was obtained by multiplying the number of flowers per cross by 50 to give a total number of eggs and then subtracting the number of functional eggs. Under field conditions, fruit of 'Bluecrop', a parent of the decaploid, averages about 50 seeds per berry. The 2 classes of eggs and 4 levels of ploidy were arranged in a 2 × 4 contingency table to determine whether the number of seedlings produced per cross was independent of the ploidy level of the male parent. The contingency X^2 was calculated according to Mather (4).

Flower buds for chromosome studies were collected from field-grown and greenhouse plants. They were fixed in 3:1 alcohol propionic acid or Newcomer's solution and stored in a freezer until examined. Meiotic observations and photomicrographs were made from smears of microsporocytes in aceto- or propionocarmine.

Pollen samples were taken of plants in the field at time of flowering and stained in propionocarmine. Prior to examination the samples were washed in 10% acetic acid in glycerine to remove excess stain. Counts of stained and unstained pollen were made in random fields under the microscope.

Results

The chromosome number of 'US 40' was determined at anaphase I to be 2n=10×=120 (Fig. 1 D).

Meiotic chromosomes showed certain interesting features. In early prophase, the chromosomes clump together in a tight mass, forming a chromatin body situated near and connected with the nucleolus. The size of the synizetic knot formed is often as small as the nucleolus (Fig. 1F). From the synizetic knot certain chromosomes emerge gradually and a series of intermediate stages could be seen (Fig. 1E). Finally, all of the chromosomes disentangle and thick threads appear (Fig. 1G). The occurrence

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of a synizetic knot in the prophase of *Vaccinium* has been discussed elsewhere by Jelenkovic and Hough (2).

Pairing of the chromosomes was scored at metaphase I. The chromosomes almost always paired as bivalents (Fig. 1A). Multivalent associations were observed in a few cells (Fig. 1B). Because of the small size of the bivalents and the tendency of bivalents to group, it was difficult to distinguish between true multivalent associations and secondary grouping of the bivalents at metaphase I. Analysis of 16 microsporocytes at metaphase I showed that only in 5 of them were multivalents present. Only 23 of the 1,920 chromosomes analyzed in the 16 microsporocytes were considered to be involved in multivalent associations. Though errors in classification of the chromosomes are possible, the fact remains that a relatively small proportion of chromosomes form multivalents.

Twenty-five microsporocytes were scored at metaphase I for univalents; all but one had univalents. They were found most often at the plates with the bivalents (Fig. 1B). In plates with bivalents in side view, an equal number of univalents was present on each side. This

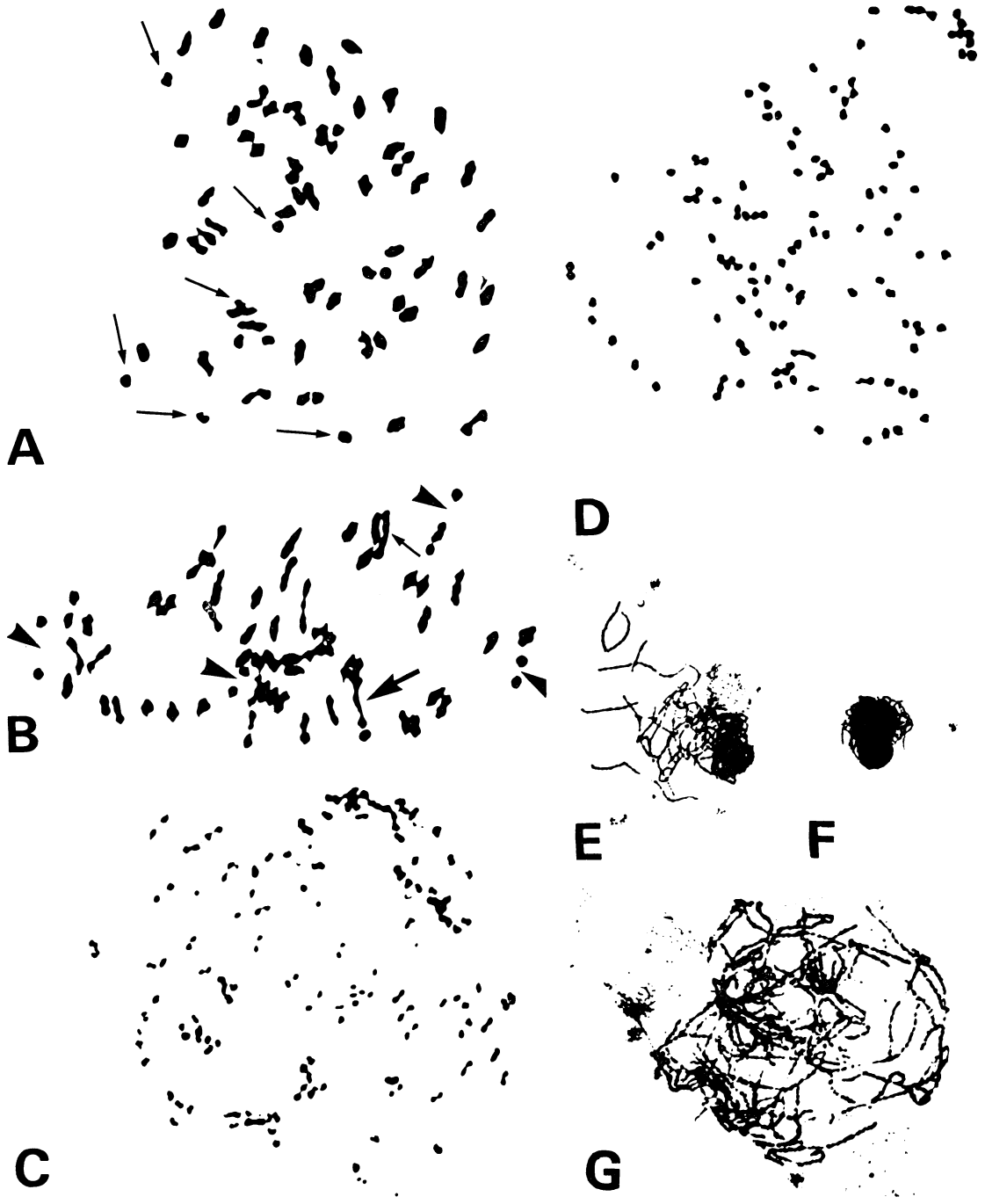

Fig. 1. First meiotic division in a decaploid blueberry. (A) Metaphase I with 57 II and 6 I (arrows). X 1,320. (B) Metaphase I with 1 VI (large arrow), 1 IV (small arrow), 51 II, and 4 bivalents precociously disjointed (arrowhead). X 1,406. (C) Early anaphase I with 32 bivalents lagging and some just disjoined. X 990. (D) Chromosome complement of decaploid, US 40, 2n = 10× = 120. X 814. (E) Pachytene threads emerging from synizetic knot. X 814. (F) Chromosomes clumped in tight chromatic mass, synizetic knot, connected with nucleolus. X 480. (G) Chromosomes at pachytene stage. X 770.

suggests that the univalents were not the result of failure of the chromosomes to pair at prophase, but rather from precocious separation of certain bivalents.

At anaphase I the great majority of the cells displayed lagging chromosomes. In only 2 of 35 cells scored at anaphase I were lagging chromosomes absent. An interesting feature of the laggards is that they are non-disjoined bivalents (Fig. 1C). A number of bivalents disjoin very early at metaphase I and separated chromosomes almost reach the poles while the rest of the bivalents remain non-disjoined. The incidence of lagging bivalents varies considerably among the cells, generally being larger in cells studied in early anaphase I than in cells in late anaphase I. The non-disjoined bivalents are rod-like in shape and no attenuation was observed (Fig. 2A, D). Cells in which the separated chromosomes at the poles were beginning to fuse, initiating the telophase stage, displayed fewer laggards and fewer disjoined bi-

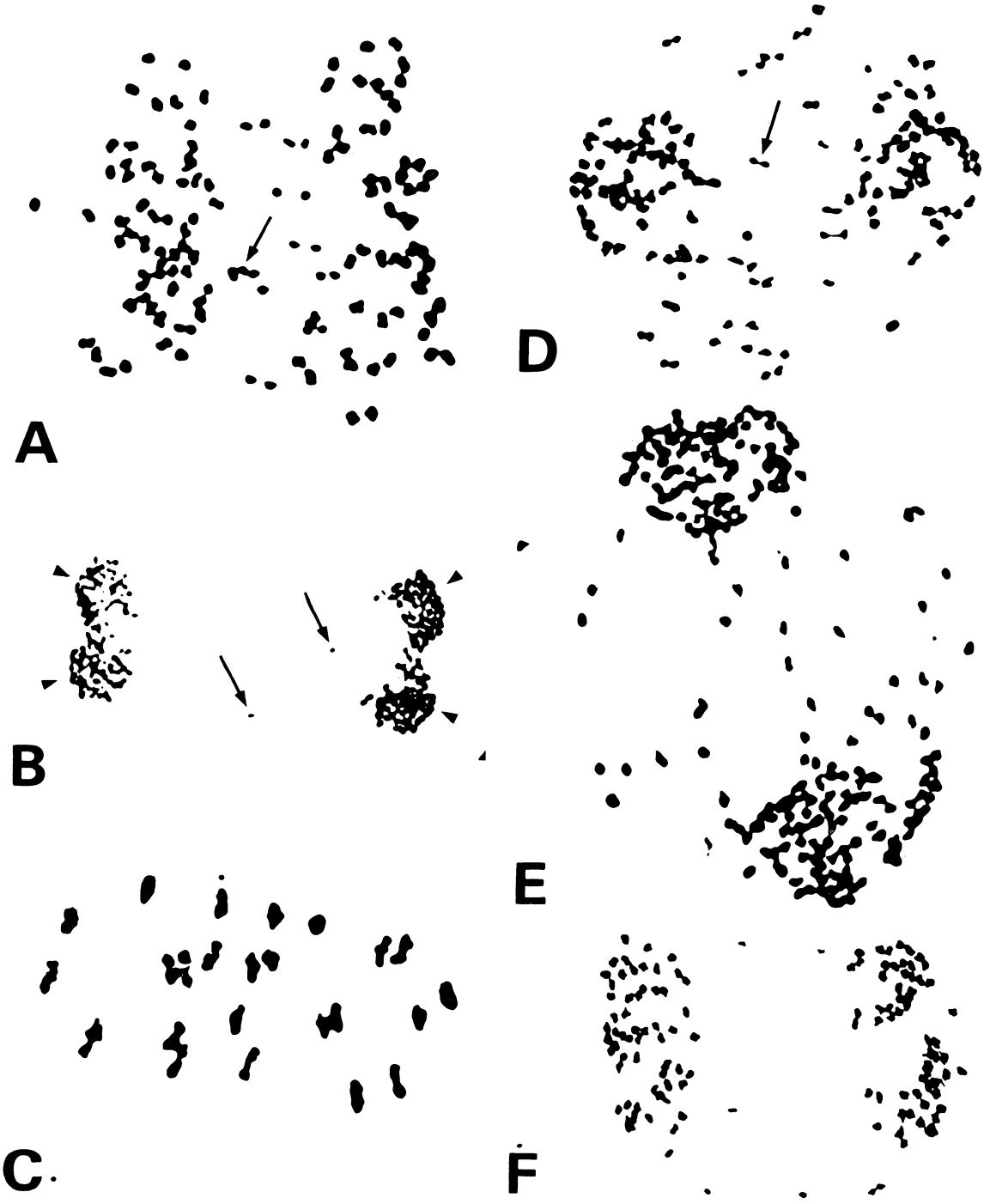

Fig. 2. Separation of the chromosomes in first meiotic division in 'US 40' and metaphase I in tetraploid 'Bluecrop'. (A) Anaphase I with 7 bivalents between disjoined chromosomes at 2 poles. In 6 of them homologous chromosomes are just separated and in one the homologues still joined (arrow). X 1,364. (B) Telophase I with 2 chromosomes not included in the group of chromosomes at either pole (arrows), and with chromosomes at poles in two groups (arrowheads). X 814. (C) Metaphase I in 'Bluecrop' with 24 bivalents regularly paired. X 1,050. (D) Anaphase I with 21 bivalents still non-disjoined between the chromosomes at the 2 poles, (arrow). X 990. (E) Anaphase I with 32 single chromosomes scattered between chromosomes at the 2 poles. X 1,365. (F) Late anaphase I with 7 single chromosomes excluded from the 2 groups of chromosomes present at each pole. X 814.

valents (Fig. 2E). Therefore, it is probable that laggards at anaphase I are caused by asynchronous disjunction of the bivalents. In a few instances, disjoined chromosomes in anaphase I were grouped at each pole into 2 groups (Fig. 2F). A similar grouping was seen occasionally at telophase I (Fig. 2B). Cells with such grouping had fewer lagging chromosomes.

In the second division of meiosis, cells were studied in anaphase II only. Of 32 cells examined, 27 showed laggards at anaphase II.

Microsporocytes of 'Bluecrop' had 24 bivalents (2n= 48) at metaphase I with regular diploid-like meiosis, though 'Bluecrop' is tetraploid (Fig. 2C).

Results of selfing 'US 40' and using it as female in crosses with diploid, tetraploid, and hexaploid genotypes are given in Table 1. There is considerable variation among and within classes of crosses in the number of seedlings produced per flower. Also, some crosses made in 1968 produced more or fewer than the same ones repeated in 1969, demonstrating a year effect. The mean number of seedlings per flower in all crosses in Table 1 was low, 0.20. The most productive pollination was the self-pollination and that averaged less than 1 seedling per flower, which is very low for blueberry.

In a test of independence in a 2 × 4 contingency table of ploidy level of male parent and functional eggs in 'US 40,' the X^2 value fell beyond the P=.001 level of probability. Thus, there are indications of association between the ploidy level of the male parent and the number of seedlings produced per flower of 'US 40'.

Results of using 'US 40' as the male parent in crosses with a number of hexaploid genotypes are presented in Table 2. The number of seedlings produced per flower among the crosses is quite variable. As with some of the reciprocal crosses (Table 1), these crosses differed in fertility between 1968 and 1969. For example, clone C of *V. amoenum* produced 0.02 seedlings per flower in 1968 and 0.42 in 1969 when pollinated with 'US 40'. The hexaploid × decaploid crosses had a mean fertility about the same as the reciprocal crosses; thus the fertility of 'US 40' is low whether used as a male or female parent.

Pollen stainability as a test of viability was used for samples of 'US 40' (decaploid), 'T-17' (pentaploid), 'Bluecrop' (tetraploid parent of T-17), and 'Tifblue' (hexaploid). 'Tifblue' is not the *V. ashei* parent of 'T-17' and 'US 40', but pollen of 'Myers' was not available. The 4 microspores resulting from microsporgenesis in *Vaccinium* remain together and each sphere is, in fact, 4 pollen grains with distinct cell walls. When one or more pollen grains abort the sphere becomes asymmetrical and the aborted portion remains colorless when stained with acetocarmine. Ninety-nine percent of the pollen of 'Bluecrop' and 'Tifblue' stained and had a uniform symmetrical appearance (Fig. 3A, B). Eight percent of the pollen of 'T-17' stained; the remainder was colorless and shriveled except for a few dyads that were present (Fig. 3C). Sixty-two percent of the pollen of 'US 40' stained and

Table 1. Number of flowers pollinated and number of seedlings produced in pollination of decaploid 'US 40' as female with pollen of decaploid, hexaploid, tetraploid and diploid genotypes.

Male parent	Year	No. flowers pollinated	No. seedlings produced	No. seedlings produced/ flower
10X				
US 40	1968	159	240	1.51
US 40	1969	356	137	0.34
				$\bar{x}$ = 0.92
6X				
V. ashei Tifblue	1968	141	3	0.02
V. ashei Tifblue	1969	205	42	0.20
V. ashei Woodard	1968	90	1	0.03
V. ashei Woodard	1969	87	3	0.03
V. ashei Fla. 6–181	1969	40	0	0.00
V. constablaei NH #C-1	1968	275	81	0.29
V. constablaei NH #C-1	1969	187	8	0.04
V. amoenum Cl. A	1968	45	1	0.02
V. amoenum Cl. A	1969	37	0	0.00
V. amoenum Cl. B	1968	53	0	0.00
V. amoenum Cl. C	1969	139	37	0.27
				$\bar{x}$ = 0.09
4X				
V. brittonii	1968	82	14	0.17
V. corymbosum	1968	89	1	0.01
V. corymbosum Darrow	1969	46	0	0.00
V. corymbosum Earliblue	1969	115	0	0.00
				$\bar{x}$ = 0.04
2X				
V. atrococcum	1969	508	30	0.06
V. myrtilloides	1969	109	18	0.16
V. tenellum	1969	219	8	0.04
				$\bar{x}$ = 0.08

Table 2. Number of flowers pollinated and number of seedlings produced in pollination of hexaploid genotypes with decaploid 'US 40' pollen.

Female parent	Year	No. flowers pollinated	No. seedlings produced	No. seedlings produced/ flower
6X				
V. ashei Woodard	1968	291	13	0.04
V. ashei Woodard	1969	93	0	0.00
V. ashei Tifblue	1968	127	2	0.03
V. ashei Tifblue	1969	280	33	0.12
V. ashei Fla. 6–186	1969	71	3	0.04
V. constablaei NH #C-1	1968	170	8	0.05
V. constablaei NH #C-1	1969	179	7	0.04
V. amoenum Cl. A	1969	49	0	0.00
V. amoenum Cl. C	1968	54	1	0.02
V. amoenum Cl. C	1969	54	23	0.42
				$\bar{x}$ = 0.07

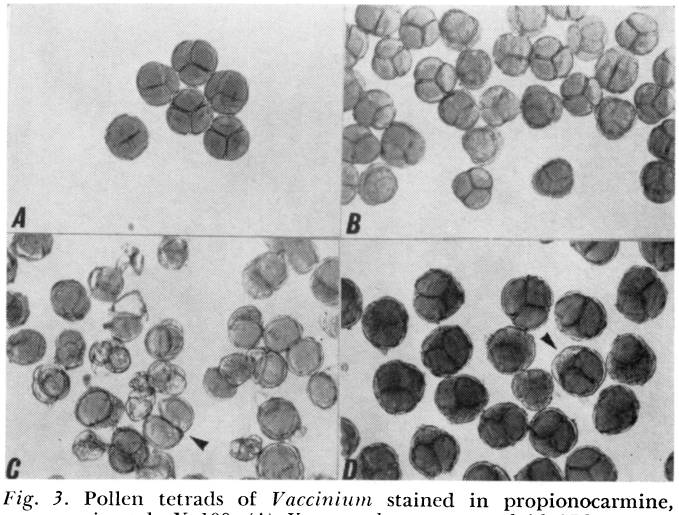

Fig. 3. Pollen tetrads of *Vaccinium* stained in propionocarmine, approximately X 100. (A) *V. corymbosum*, tetraploid ('Bluecrop'), pollen uniform in size and staining. (B) *V. ashei*, hexaploid ('Tifblue'), pollen uniform in size and staining. (C) Pentaploid, hybrid of *V. corymbosum* × *V. ashei*, pollen irregular in size and stainability with some dyads (arrow). (D) Pollen of decaploid showing large size and unstained shriveled grains (arrow) of some tetrads.

appeared symmetrical (Fig. 3D). The fertility of 'US 40', as judged by pollen appearance, was higher than that of the pentaploid, 'T-17', but was less than that of the cultivated tetraploid and hexaploid genotypes.

Discussion and Conclusions

Cytological evidence indicates that most of the microsporocytes of 'US 40' (decaploid) had irregular chromosome distribution at anaphase I and anaphase II. Such chromosome distribution results in formation of aneuploid gametes. Because of the probable duplication of the genetical material in the decaploid, gametes with aneuploid chromosome number would be expected to be functional and therefore seed setting would not be seriously hampered. However, low fertility of the crosses indicates that the unbalanced gametes are probably nonfunctional. This is supported by the evidence that about 1% of the gametes were functional when the decaploid was self-pollinated. High incidence of aborted microspores in 'US 40' supports the same conclusion. In crosses of 'US 40' with hexaploid, tetraploid and diploid genotypes, we expected higher fertility, since the male gametes are of balanced chromosome constitution. This was not the case in these crosses, and therefore low fertility cannot be attributed solely to irregular chromosome distribution in the first and second meiotic divisions. The same conclusion is supported by results of crosses of 6X $\times$ 10X. By using 'US 40' in crosses with heteroploid male parents, the ratios of basic complements in zygote, endosperm and surrounding somatic tissue change. Thus, in the case of 10X $\times$ 10X cross these ratios are: 1:1.5:1, whereas in crosses 10X $\times$ 2X and 6X $\times$ 10X the ratios become: 1:1.83:1.66 and 1.33:1.83:1 respectively. Stebbins (6) indicates that differences in results of reciprocal crosses with heteroploid genotypes were caused by altering these ratios. At present, we do not have any indication at what stage after pollination the failure occurs in blueberry.

The inconsistency in number of seedlings obtained was found to be a general phenomenon, even with the pollen of the same genotype in different pollination seasons. Perhaps, in these polyploids, the delicate metabolic steps related to fertilization are easily disturbed either by heteroploid male gametes or by environmental conditions resulting in frequent seed abortion. From a practical view, however, large numbers of flowers of the decaploid must be pollinated per cross in order to have populations large enough for effective selection of superior types.

In this decaploid, practically all of the chromosomes pair as bivalents. Perhaps the chromosomes derived from the tetraploid and hexaploid in the pentaploid are not homologous, and after doubling, each chromosome has an identical homologue, thus normal pairing and bivalent formation are restored. Although detailed study of chromosome pairing in the pentaploid has not yet been completed, our observation, so far, indicates that only 7 to 12 chromosomes are not paired in each microsporocyte. On the basis of this observation, four-fifths of the chromosomes in the decaploid or 96 chromosomes would be expected to form quadrivalents occasionally. The proportion of the chromosomes involved in multivalent associations, however, is much lower. The relatively low incidence of the quadrivalents is probably due to preferential pairing of the identical chromosomes after doubling. Darlington (1) has established that bivalents can disjoin asynchronously at metaphase I. It is generally believed that the lagging of bivalents at anaphase I is caused by interstitial chiasma formation and its failure to terminalize. In hybrids, presumably because of the structural differences in the chromosomes, the process of terminalization of chiasmata is arrested and such bivalents lag in anaphase I. However, in the case of structural hybridity the bivalents often become attenuated at anaphase I (3). As we have shown, no attenuation of bivalents was determined in the preparations of the decaploid and we are inclined to believe that the lagging bivalents resulted from pairing of identical partners. Structural hybridity, therefore, probably does not account for lagging bivalents at anaphase I.

The original pentaploid was produced by crossing tetraploid 'Bluecrop' *(V. corymbosum)* with hexaploid 'Myers' *(V. ashei)*. The tetraploid has 3 different tetraploid species in its genetic background. This means that, after doubling, 72 chromosomes of the decaploid were from *V. ashei* and presumably the other 48 tracing from three different genomes. It is apparent that genomic make-up of the decaploid is a rather complex one. As Darlington (1) pointed out, chiasma formation and terminalization are characteristic properties of each species. It is possible then, that chromosomes from certain genomes incorporated in the decaploid are 'out of phase' with the others during disjunction of the bivalents in meiotic division.

The cause and mechanism of the grouping of the chromosomes observed at anaphase and telophase I are not known at present. It is probable that this unusual chromosome behavior is caused by irregularities in spindle formation.

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