June M. Vail

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FREDERICK V. COVILLE

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A. E. LONGLEY

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BLUEBERRY CHROMOSOMES

FOR sixteen years the writer has been making experiments in the hybridization and selection of native American blueberries, species of the genus *Vaccinium*. The practical outcome of these experiments has been the development of valuable horticultural varieties producing berries of very large size. Some of the hybrids now in commercial cultivation, Pioneer, Katharine, Cabot and Rancocas, have yielded berries three-quarters of an inch in diameter, and last year the berries of two unnamed hybrids, 1257A and 1443A, reached a diameter slightly in excess of seven-eighths of an inch.

In the course of these experiments it was found that certain species, some of them very different in general appearance and in technical characters, hybridize readily. Other species, some of them very closely related, are sterile to each other's pollen and yield no hybrids.

The lowbush blueberry, *Vaccinium angustifolium*, of the northeastern United States, hybridizes easily with the highbush blueberry of the same region, *V. corymbosum*. The horticultural variety Greenfield is a second-generation hybrid between these two species, containing two quarter-strains of each. The horticultural variety Rancocas is another second-generation hybrid of the same ancestry, containing one quarter-strain of lowbush blueberry and three quarter-strains of highbush blueberry. Natural hybrids between these two species are of frequent occurrence in New England pastures. The plant named in Gray's Manual *Vaccinium corymbosum amoenum* is one of these natural hybrids.

The dryland blueberry (*Vaccinium vacillans*), the Canada blueberry (*V. canadense*), and the bigbush

blueberry (*V. atrococcum*) could not be crossed with either the lowbush or the highbush blueberry. That the highbush and the bigbush blueberry did not hybridize was to me very surprising, for the two species are closely related, so closely indeed that Asa Gray regarded one as a variety of the other.

Two southern species, the hairy blueberry, *Vaccinium hirsutum,* and the myrtle blueberry, *V. myrsinites,* hybridize freely in the greenhouse with both the highbush and the lowbush blueberry, notwithstanding the great structural differences between the species thus hybridized. One, *Vaccinium hirsutum,* a species of the southern mountains, has a bristly-hairy fruit, inconspicuous winter flowering buds, an extraordinarily large stigma, and almost woolly leaves. The other, *V. myrsinites,* a species of the southern coastal plain, has very small evergreen leaves, and hardly looks as though it belonged to the same genus as the highbush and the lowbush blueberry of the north. That these strikingly different southern species hybridize easily with the two northern species surprised me greatly.

Desiring to learn the reason for this curious grouping of blueberry species, with reference to hybridization, I tried for several years to induce some one of the plant cytologists to make a study of blueberry chromosomes, but without success. Cytologists are scarce and busy. At last, through the mediation of G. N. Collins, Dr. A. E. Longley undertook the sport of hunting the blueberry chromosome. He began the work in the spring of 1924 and continued it in the years following, as material became available. Dr. Longley's results up to this time are presented in a paper accompanying this paper of mine.

Dr. Longley has made the discovery, important and significant in blueberry breeding, and fascinating in the facility with which it removes obstacles to this

pursuit, that certain species of blueberry have twelve chromosomes, others twenty-four, and still others thirty-six.

The highbush and lowbush blueberry, which hybridize freely, both in nature and artificially, have twenty-four chromosomes. In the dryland blueberry, the Canada blueberry, and the bigbush blueberry, no one of which has hybridized with either the highbush or the lowbush blueberry, the number of chromosomes is twelve.

The question naturally arose whether the 12-chromosome species would not hybridize with each other. It happened that these crosses had never been attempted, because in these three species no plants had been found whose characteristics were desirable for combination. With the new incentive, however, numerous cross-pollinations were made in 1926 between the Canada blueberry and the dryland blueberry and between the dryland blueberry and the bigbush blueberry. Fruit set promptly, the berries contained an abundance of seeds, and the seeds have now produced vigorous young plants, some of them ready to flower next spring.

From still another cross, not yet mentioned, curious results had been obtained. This was a cross, made in 1922, between the rabbiteye blueberry of Florida, *Vaccinium virgatum,* and one of the large-berried northern hybrids. Many of the pollinations failed, but berries containing seeds were obtained in sufficient number to produce several hundred seedlings. They grew with great vigor and flowered freely, but although hundreds of pollinations were made on them with pollen of known virility, not a single well-developed berry resulted, and the occasional small and late berries they bore contained no seed possessing an embryo. In the production of offspring this cross, therefore, has proved completely sterile.

Upon examining the rabbiteye blueberry, Dr. Longley found that this species has thirty-six chromosomes. The plant with which it was crossed has twenty-four chromosomes. The resulting sterile hybrids usually have thirty chromosomes.

Since many who read this paper are doubtless unfamiliar with the action of the chromosomes, the minute bodies that are reputed to carry to the offspring the characteristics about to be inherited from the two parents, the following brief statement is presented regarding them. It represents the ideas current among geneticists. When the first cross-pollination in this series was made, the thirty-six chromosomes from the pollen grain of one parent were poured into the egg cell of the other parent, which already contained twenty-four chromosomes. The total of sixty chromosomes was carried through each cell of the resulting hybrid, in the ordinary process of cell division, until the plant was nearly ready to flower. Then ensued a phenomenon known as the reduction of the chromosomes, in the cells that produce the pollen grains and the egg cells. Presumably twenty-four of the sixty chromosomes, representing those derived from the 24-chromosome parent, combined with twenty-four of the thirty-six chromosomes representing the other parent. The remaining twelve chromosomes from the second parent, having no chromosomes with which to pair normally, paired abnormally with each other or remained unpaired. This abnormal pairing of the chromosomes, according to the current view, caused a derangement of the normal activities of the plant, which resulted in sterility of fruit production.

The rabbiteye blueberry has come into cultivation extensively in the South by the transplanting of the wild bushes. It is of great importance that this species be improved by hybridization. The first attempt

to do this failed, seemingly because the rabbiteye blueberry stood alone in the number of its chromosomes. The possibility of improvement appeared to depend on the finding of another species having thirty-six chromosomes, and possessing also desirable characteristics that could be transmitted to a hybrid.

In the higher Appalachian mountains of western North Carolina and eastern Tennessee occurs a native species, *Vaccinium pallidum,* the Blueridge blueberry, which has large, beautiful and delicious fruit. As early as 1911, attempts were made to cross this with the highbush blueberry and the lowbush blueberry; but all the pollinations failed, and the Blueridge blueberry was therefore abandoned as a breeding stock.

In the hope that this blueberry might be a 36-chromosome species, because it had failed to hybridize with the 24-chromosome species, plans were made, for the spring of 1927, to determine its chromosome number. Material was obtained from western North Carolina through the courtesy of George E. Murrell, horticulturist of the Southern railway. On critical study of the material Dr. Longley found, to the great delight of all of us, that the Blueridge blueberry has thirty-six chromosomes.

If future experience confirms the view that the number of the chromosomes in blueberry species is a true index of the facility of their interbreeding, as the experiments indicate thus far, we shall be able next spring to hybridize the rabbiteye blueberry with the Blueridge blueberry, and thus add one more item to our knowledge of the means by which wild species become plastic in the hands of science.

FREDERICK V. COVILLE

U. S. DEPARTMENT OF AGRICULTURE

CHROMOSOMES IN VACCINIUM

A CYTOLOGICAL investigation of the number of chromosomes in a dozen *Vaccinium* species and hybrids has revealed three diploid, six tetraploid, one pentaploid and two hexaploid forms.

The material used for this study of the chromosome in microspore-mother-cells was collected early in the springs of 1924, 1925, 1926 and 1927 from *Vaccinium* plants grown under the direction of Dr. Frederick V. Coville at the greenhouses of the Bureau of Plant Industry, Washington, D. C., and from plants growing in their wild habitats.

Two methods were used in preparing buds for study. In one, the buds were killed with chromo-acetic killing fluid, embedded in paraffin and stained with Haedenhains haematoxylin. In the other, fresh collected buds or buds killed in acetic-absolute (1–3) were stained with aceto-carmine killing and staining fluid. The former more tedious method was very satisfactory and served as a check against the latter quicker method, which was found to give excellent preparations when the difficulty of using aceto-carmine on the minute anthers of *Vaccinium* was overcome.

DIPLOID SPECIES

Vaccinium atrococcum, wild plant from Aurora Hills, Va.
Vaccinium canadense, wild albino plant (Shear).
Vaccinium canadense, wild plant (La Roche).
Vaccinium vacillans, wild plant from Aurora Hills, Va.
Vaccinium vacillans, wild albino plant from New Jersey (MacIlvaine).

These three species were found to have 12 bivalent chromosomes at diakinesis of the pollen-mother-cell. Since 12 is the lowest number found in any *Vaccinium* species, it seems probable that 12 is the basic number for the genus, and that the three forms listed

are true diploid species. This view is substantiated from our study of *Polycodium stamineum* and *Gaylussacia baccata,* representative forms of two closely related genera. The same basic chromosome number, 12, characterizes these two species.

The meiotic phases in the pollen-mother-cells of these three species are passed through in a very regular manner, giving each cell of the tetrad the reduced chromosome number. Text figure 1A shows the chromosomes of *Vaccinium canadense* in the heterotypic prophase. The chromosomes are small compact masses at this phase and show no individual morphological characteristics.

Tetraploid Species

Vaccinium angustifolium, wild plant from Middlesex Fells, Mass.
Vaccinium angustifolium, wild plant (Russell).
Vaccinium corymbosum, wild plant from Lincoln, Mass.
Vaccinium corymbosum, wild plant (Taylor No. 2).
Vaccinium corymbosum, wild plant from North Carolina (Sampson).
Vaccinium hirsutum, wild plant.
Vaccinium corymbosum × *V. corymbosum* (Dunfee × Rubel, plant No. 20 of culture 2300).
Vaccinium angustifolium × *V. hirsutum* (culture 1560).
Vaccinium angustifolium × *V. myrsinites* (culture 1535).
(*Vaccinium angustifolium* × *V. myrsinites*) × *V. corymbosum* (culture 1908).

The three species and four hybrids listed above were found to have 24 as the reduced or haploid number of chromosomes.

The increase from 12 to 24 chromosomes made it more difficult to find cells where chromosome counts could be made. Text figure 1B shows the chromosomes at diakinesis in *V. angustifolium* (Russell). The chromosomes show to some extent their paired nature at this stage. A few assume such shapes as

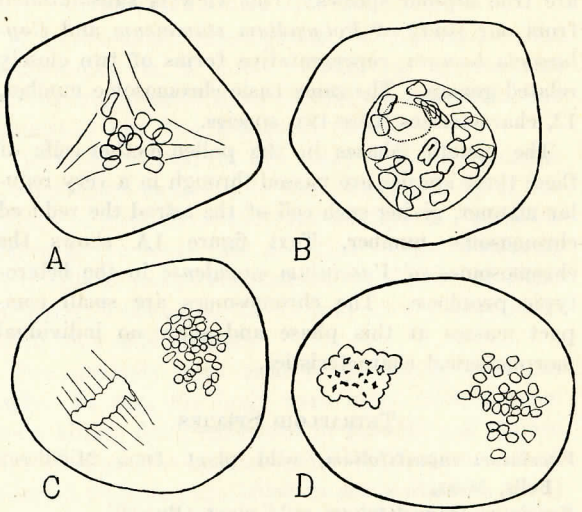

FIG. 1. Microspore-mother-cells of *Vaccinium*: A, heterotypic prophase in *V. canadense*, showing spindle and twelve chromosomes; B, diakinesis in *V. angustifolium*, showing twenty-four bivalent chromosomes; C, homotypic metaphase in *V. pallidum*, side view of left spindle, end view of right spindle, the latter showing thirty-six chromosomes; D, homotypic metaphase in *V. corymbosum* × *V. virgatum*, the plate at the right showing thirty chromosomes. (A, × 800; B, × 800; C, × 600; D, × 500.)

opened and closed rings but a detailed study of individual chromosome characters was not attempted.

HEXAPLOID SPECIES

Vaccinium virgatum, wild plant from Crestview, Florida (culture 1881).

Vaccinium pallidum, wild plant from Pisgah Ridge, North Carolina.

In the spring of 1926 some buds from two plants of *V. virgatum* were procured. In this species 36 haploid chromosomes were found.

The discovery of a hexaploid species led us to extend our search. In the spring of 1927 buds of *V. pallidum* were procured. Our material gave us only a few well-preserved cells in which the chromosome number could be counted. Fig. 1C pictures the homotypic metaphase of *V. pallidum* showing 36 chromosomes.

Diploid, tetraploid and hexaploid forms all show a regular pairing of chromosomes at diakinesis and abnormalities were very rare in any of the reduction phases.

Pentaploid Hybrid

Vaccinium corymbosum × *V. virgatum* (Katharine × Rabbiteye).

The reduction stages were studied in several F_1 plants of the foregoing interspecific hybrid. Each showed abnormalities such as are usually met with in hybrids whose parents had different chromosome numbers. Occasionally all chromosomes were paired, giving bivalent chromosomes at diakinesis. A regular mother-cell is pictured in Fig. 1D, in which there are 30 chromosomes. More frequently the mother-cells are found to be much vacuolated and the reduction phases irregular, giving as a result polycary, polyspory and very little normal-appearing pollen.

A. E. Longley

U. S. Department of Agriculture