A NOVEL TYPE OF HYBRIDITY IN FRAGARIA

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INTRODUCTION

In 1930 I described briefly a case of hybridity in which apparently there had been a union between an egg cell of *Fragaria vesca* L. ($n=7$) from Hawaii and a single genom ($n=7$) of *Fragaria virginiana* Duchesne ($n=28$) from the eastern part of the United States. The variety of *F. vesca* used as the female parent had white fruit. This plant was isolated in a room containing no other plants and was pollinated with pollen from a plant of *F. virginiana* having red fruit. Several hybrids were obtained having 35 chromosomes. They resembled *F. virginiana*. One plant only was found which had 14 chromosomes. Any strawberry specialist would have said that it was a plant of *F. vesca*, for it had all the major characteristics of this species. (In my preliminary paper, I stated that this plant resembled *F. vesca* in every respect, and then contradicted myself by saying that it showed "several other characteristics of *F. virginiana*." The phrase was "minor characteristics" in the manuscript, but the word "minor" was left out by mistake. I had not, at that time, noted the margin difference in the middle leaflet.) The differences noted were red fruits (not a specific distinction), rootstocks slightly stouter, and leaflets slightly firmer and darker in color (as in *F. virginiana*) but not beyond the limits of variation of other varieties of *F. vesca*. It was also found that the center leaflet was dentate only halfway to the petiolule—as is characteristic of the variety of *F. virginiana* used—instead of being dentate for three-fourths of the distance, as in the maternal variety.

The phenomenon was interpreted as above. It was thought to be similar to the "maternal," but red-fruited, type discovered by MILLARDET (1894) after an attempt to cross a white-fruited *F. vesca* with pollen from a red-fruited *F. chiloensis*. Later, it occurred to me that since the plant of *F. virginiana* which furnished the pollen had not been isolated, as was the mother plant, and since there were a few red-fruited plants of *F. vesca* in flower in another part of the greenhouse, there was a remote possibility that the plant in question was the result of crossing two varieties of *F. vesca*.

During the past four years, the original plant has been studied carefully, together with numerous clones. In addition, the behavior of 18 F_2 plants has been investigated. From the evidence thus obtained, there is little room to doubt the correctness of the original interpretation.

EXPERIMENTAL WORK

Observations on the supposed hybrid and the parents

Among the various species of the genus *Fragaria*, even those with different chromosome numbers are difficult to classify. By experience one learns to distinguish the various clones of a given group; but the criteria used are largely quantitative rather than qualitative. No one who has worked with strawberries is unable to separate *F. vesca alba* from *F. virginiana* without looking at the color of the fruit; yet when the descriptions are set down in words, the distinguishing marks mean little to those who are not familiar with the material. At the same time, it should be noted that the descriptions of *F. vesca alba* and *F. virginiana*, as set down in table 1, exhibit differences that are really decisive individually, since in most cases they do not overlap.

TABLE 1

Characteristics of F. vesca alba, F. virginiana, and the supposed diploid hybrid.

CHARACTER	F. VESCA ALBA	SUPPOSED DIPLOID HYBRID	F. VIRGINIANA
Chromosome pairs	7	7	28
Rootstock	slender	slightly stouter	medium stout
Runners	long	long	medium
Runners	light red	light red	red
Hairs	profuse, spreading	profuse, spreading	shorter, less spreading
Petioles	long, slender	long, slender	shorter, medium stout
Leaflets	almost sessile	almost sessile	slightly stalked
Center leaflet	2/3 dentate	1/2 dentate	1/2 dentate
Leaflets	light green, soft	light green, stouter	green, stiff
Scapes	strong, long, erect	strong, long, erect	strong, medium pendulous
Flowers	herm., small, white	herm., small, white	herm., medium, white
Calyx lobes	spreading or reflexed	spreading or reflexed	constricted
Stamens	short	short	long
Pollen	92 percent good	63 percent good	89 percent good
Fruit	conical, white	conical, red	shorter, red
Fruit	subacid	subacid	acid
Fruit flesh	soft	soft	firm
Achenes	superficial	superficial	sunken

Populations were raised from selfed seed of each of the pure species. The individual plants showed very little variability.

About 50 plants were also raised from runners of the original hybrid. They were as much alike as if they had been cut out with a die. Nine blossoming periods were observed. The flowers that came in the spring showed an average of 63 percent of good pollen as observed in aceto-carminic smears. Those which appeared in the winter had an average of 51 percent of good pollen. These figures should be compared with 92 per-

cent and 87 percent, respectively, for *F. vesca alba* and 89 percent and 84 percent, respectively, for *F. virginiana*. The ovules of the supposed hybrid all appeared to be functional, since berries perfectly filled with seeds were obtained. But the hybrid had one distinguishing feature apart from a marked heterosis and the points already mentioned. *The anthers did not dehisce except rarely*. Left to themselves the plants seemed to be almost completely sterile. When the anthers were scraped with a fine scalpel and the flowers were pollinated by hand, however, good fruits were obtained.

To sum up, then, the supposed hybrid was like the maternal parent, *F. vesca alba*, in all characters, except that it had red fruits, less extended leaflet dentation—particularly in the center leaflet—and somewhat stouter rootstocks and runners. It showed marked heterosis, had a much greater percentage of pollen abortion than either parent, and was almost lacking in the power to dehisce.

Observations on the F₂ generation

Ordinarily, selfed seeds of diploid varieties of *Fragaria* have germinated in percentages varying between 70 and 85. Three populations have been recorded in our experience where the percentage of seed germination was less than 60. The lowest percent was 43. *In the case of the supposed hybrid, two tests gave the figures 15 percent and 11 percent, respectively.*

Eighteen plants have been raised to maturity and have been observed continuously for over three years since that time. This duration of time included 6 blossoming periods for some of the plants. This was true of No. 8 and No. 11, both being fertile. In addition, Nos. 3, 4, 5, 7, 8, 11, and 18 blossomed from 1 to 4 times and showed a reasonable degree of fertility by producing moderate quantities of fruit. The pollen, under aceto-carmin, varied in normality from flower to flower and even from anther to anther, as well as from plant to plant. The limits of variation were 39 percent and 79 percent. Dehiscence was fair, but not wholly normal. There were 5 red-fruited and 2 white-fruited plants (Nos. 7 and 8).

Plants 2, 16, and 17 did not flower during the three years that they were under observation as mature individuals. This is something that had never happened before in our experience with diploid *Fragarias*, though it had been comparatively common in the dwarf hybrids arising from the union of plants having different chromosome numbers.

Plants 1, 9, 10, 12, and 13, which blossomed once, and plants 6, 13, and 14 which blossomed twice, were completely sterile. Under aceto-carmin examinations, from 5 percent to 30 percent of the pollen of these plants appeared to be viable; but even with hand pollination no fruits were obtained. Only rarely did an anther dehisce.

These eighteen plants exhibited an amount of variation that was far beyond anything that we had seen among diploid *Fragarias*. No. 13 was

FIGURE 1.—Representative leaves of *Fragaria vesca alba*, *Fragaria virginiana*, the 14-chromosome F_1 plant, and plants 1–14 of the F_2 generation.

a dwarf, extremely compact; Nos. 1, 5, and 14 were very compact; Nos. 12 and 18 were very loose and straggly in habit; the remainder varied in

size, but were within the limits of variation of the diploid type of *Fragaria*.

Plants 12 and 15 (sterile) had twenty-one chromosomes, the remainder had fourteen chromosomes. These counts were made by my technical assistant, Miss ESTELLA HUMPHREY, from root-tip preparations. I had the opportunity of studying PMC slides from only two plants—both being of the diploid type. In both there was much more irregularity than I have seen in similar preparations of pure diploids made by Mr. ICHIJIMA and by Dr. YARNELL in this laboratory.

The variability of the plants in other decisive characters was as follows.

In the type of rootstock developed, 10 were slender, as in *F. vesca*, 4 were intermediate, and 4 were stout, as in *F. virginiana*.

FIGURE 2.—Representative leaves of plants 15–18 of the F_2 generation.

In 10 plants the runners were as slender as those of *F. vesca*; in 5 plants they were as stout as those of the *F. virginiana* used; while in 3 plants they were much stouter than those of the *F. virginiana* used. There were 11 plants with long, 6 plants with medium, and 1 plant with short runners. And there was segregation in the two cases. The slender runners were not always long. In fact, in the plant where the runners were very short, they were also very slender. In 12 instances the runners were red or dark red, as in *F. virginiana*; while in 6 cases they were very light red, as in *F. vesca*.

The shape of the leaves, the dentation, the length of the dentate portion, the size of the stipules, the length of the petioles, and the length of the petiolules of each plant of the F_2 generation, together with the same characteristics for the parents and for the F_1 plant are shown in figures 1 and 2. We have found that the length of the petiolules and the length of

the dentate portion of the center leaflet form very critical diagnostic characters. The variation shown in the figures is much greater than that found in populations of either parental type. The leaf drawn is as near an average for the plant as one could determine; but of course a consideration of each plant as a whole gives a better idea of the amount of variation existing. Two important leaf characteristics not shown by the drawings are color and stiffness. There is a high degree of correlation between the two. In 10 plants the leaves were soft and light green; in 4 plants the leaves were dark green, tending to glossiness, and stiff. In only 1 diploid, No. 11, were the leaves dark green yet soft. The leaves of the triploids were dark green, very stiff and rugose.

In *F. vesca*, the scapes are long and erect; in the *F. virginiana* used, they are short and drooping. Of the fifteen F_2 plants which blossomed, 3 had long erect scapes, 2 had long drooping scapes, 5 had short scapes similar to *F. virginiana*, and 5 had very short scapes.

The flowers of each plant were hermaphroditic, 5 being much smaller than those of *F. vesca*, and 9 being about the size of the flowers of that species. Only 1 plant had flowers approaching the size of the putative male parent. All the plants but one had small concave petals as in *F. vesca*.

It is characteristic of *F. vesca* to have a spreading calyx in which the lobes are reflexed. In *F. virginiana*, on the other hand, the calyx is usually constricted, often closing around the base of the maturing fruit. This character was difficult to score on the F_2 plants by reason of the great amount of sterility shown, but there were only 2 plants that showed a tendency toward a constricted calyx.

One of the best diagnostic characters is stamen length. They are short in *F. vesca* and long in *F. virginiana*. Among the F_2 plants there were 10 with short stamens, 1 with stamens of medium length, and 4 with long stamens. In 5 plants the stamens were almost sessile—an extreme variation that I had not seen before.

The 7 plants that fruited (5 red, 2 white) had berries which were conical with long necks like those of *F. vesca*. The achenes were set superficially, except in one instance. There they were pitted as in *F. virginiana*. The flavor of all the fruits was subacid, and the flesh was mealy.

DISCUSSION AND SUMMARY

Let us recapitulate the more important facts and arguments involved in this study.

An isolated plant of *F. vesca alba* ($n = 7$) was pollinated with pollen from a plant of *F. virginiana* ($n = 28$) from the eastern United States. Several normal hybrids with 35 chromosomes were obtained. In addition there was a plant having 14 chromosomes that resembled the maternal parent

except as follows: noticeable heterosis, markedly lower pollen fertility, non-dehiscing anthers, red fruits, smaller amount of dentation on center leaflet, and stouter rootstocks and runners (the last two characteristics possibly due to heterosis and, at all events, unimportant).

The possible explanations of the origin of this plant are: (1) mutation; (2) contamination of the pollen of *F. virginiana* with the pollen of a red-fruited *F. vesca*, and (3) fertilization of the *F. vesca* egg cell having 7 chromosomes with a 7-chromosome genom from *F. virginiana*—the remaining 21 chromosomes being extruded into the cytoplasm and lost.

Obviously, the facts presented do not admit of the mutation interpretation. Moreover, there is no known instance of a white-flowered variety's giving rise to a red-fruit (dominant) mutation, although this fact does not preclude the possibility of such a mutation. We have to decide between interpretations two and three.

The characteristics of the F_1 plants (asexual reproduction) do not force a decision, though the non-dehiscing anthers and the greatly increased percentage of sterile pollen make one inclined to accept interpretation three. I say this in spite of the fact that a study of meiosis in this material gave us no clue as to the cause of the increased sterility. The P. M. C.'s appeared to go through their divisions normally.

The behavior of the eighteen F_2 plants, and the nature and extent of the variability which they exhibited, however, do force us, I think, to accept interpretation three. From previous experience, we know that plants of *F. vesca alba* crossed with pollen of the two similar red-fruited varieties that were in blossom at another part of the greenhouse when the flowers were taken from *F. virginiana* to make the cross under discussion, always flower, never show increased pollen sterility, and exhibit only a minor amount of variability. The variability found is that characteristic of an increased or decreased food supply. It affects the diagnostic characters used by taxonomists (noted in the text) so slightly that only a student of the genus *Fragaria* would notice the changes. And in no case do several diagnostic characters pass the limit of specific variability at one time.

In this small F_2 population of eighteen plants, it should be noticed, all the above phenomena occur. There are two triploids; three plants go through three years of growth after maturity without blossoming; eight plants are sterile; and variability exceeds specific limits in such diagnostic characters as the length of the petiolules, dentation of the center leaflet, length of the scape, size of the flower, and length of the stamens, besides showing an extraordinary variability in characters that have less specific constancy.

One may ask how such an odd case of hybridity could arise. On this

point a study of the maturation divisions in the pollen mother cells of some true hybrids between *F. vesca* and *F. virginiana* has yielded some information. In these thirty-five chromosome hybrids seven pairs are nearly always found, though there is sometimes a little irregularity. The remaining twenty-one chromosomes are commonly thrown out into the cytoplasm. Apparently the seven-chromosome gametes, which presumably are sometimes formed, are not functional—possibly because of certain imaginable cytoplasmic restrictions on functionability. But there seems to be no good reason why the fertilized egg cell in such a case should not eliminate the excess of chromatin and go through the divisions of ontogeny as a fourteen-chromosome plant.

If this interpretation is accepted for the novel hybrid described here, the phenomenon forms the basis for some very interesting reflections. The *Fragarias* are widely distributed. They exist in the wild as diploids, hexaploids, and octoploids. The limits of variation in the genus are narrow. The diploids are so similar that the experts do not agree on their classification. The hexaploids and octoploids are hardly more differentiated from the diploids than are the polyploids which have arisen from diploids in the genetic laboratory. That is to say, the octoploid *Fragarias* are as similar to *F. vesca* and its relatives as the tetraploids of *Oenothera* and *Primula* are to the diploids which gave rise to them. *Fragaria* is, then, a very constant genus, and one which might be expected to show a high degree of genetic compatibility among its members. Here there is a Hawaiian diploid so similar in gene constitution to an American octoploid that a genom from the latter will fit fairly harmoniously into the business of development when paired with a genom from the former species. Even meiosis in the hybrid exhibits no marked irregularity. At the same time, it should be noted that in the segregation which occurs in the F_1 generation, gene combinations are formed which do not fit so well. The result is plants which do not blossom and plants which are sterile when they do blossom.

LITERATURE CITED

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