GENETICAL STUDIES IN APPLES. II

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(With Two Text-figures)

Extensive breeding experiments have been carried out with apples, but little precise knowledge of their genetics has been obtained. This is mainly due to the apparent continuous variation of many characters investigated. Further, their heterozygous constitution and the prevalence of self-sterility prevents genetical studies from being directly approached.

In a previous report on these experiments (Crane & Lawrence, 1933) an account was given of genetical studies of fruit characters, such as colour and russetting of the skin, season of ripening, shape and size of fruit. Similar studies have been reported upon by other investigators, notably Wellington (1924) and Wilcox & Angelo (1935), and with one doubtful exception simple disomic segregation was not found in any of the characters studied.

Other characters, such as leaf shape and petiole length (Tydeman, 1935) and the formation of root burrs appear to be quantitatively inherited. With respect to albinism Crane & Lawrence (1933) obtained results which closely approximated to a 3 : 1 ratio. However, such a ratio could be expected for either disomic or polysomic inheritance when the genes in a polyploid are in the simplex condition.

In general it was concluded from the above investigations that inheritance in apples was polysomic and that a number of genes were involved in the expression of the characters studied. This to some extent supports the theory advanced by Darlington & Moffett (1930) that cultivated apples are secondary polyploids, the so-called diploids ($2n = 34$) being in part tetraploid and in part hexaploid.

The character under investigation to be described in this paper is the anthocyanin pigmentation of leaves and shoots. The anthocyanin coloration is a convenient character for genetic study, as it develops in the early seedling stage and the plants can be scored and the majority discarded while quite small, thus obviating the labour and expense of growing trees to maturity. The investigation is incomplete, but since to complete a genetical analysis would require at least another generation of plants, which would take seven years, this preliminary report is

published. In addition a brief account is given of a technique used for increasing the proportion of seeds which germinate. This may be of value when applied to other plants where under normal treatment many seeds fail to germinate.

The experiment was begun in 1920 by crossing "Lord Grosvenor", a variety of *Malus Malus*, which has normal green leaves, with *M. Niedzwetzkyana*, in which the leaves and all parts of the tree are deeply pigmented with anthocyanin. Some of the F_1 plants began to flower in 1929, and two of them, with four other green-leaved varieties,

TABLE I

	Shoots	Leaf	Flower petals	Fruit
Malus Niedzwetzkyana	Dark purple, wood purple	Tinged red, turning purple in autumn, petiole and midrib bright red	Deep rose purple	Skin crimson purple, flesh rose purple
Lord Grosvenor	Green with red tinge, wood unpigmented	Pale green	White with a tinge of red	Skin pale yellow, flesh white
Cox's Orange Pippin	Red, wood unpigmented	Green, petiole tinged red when young	Upper side white, lower side pale pink	Skin yellowish, flushed and stippled dull red, flesh creamy yellow
Emneth Early	Green with red tinge, wood unpigmented	Dark green, petiole with tinge of red	Upper side white, lower side pale pink	Skin yellow, flesh cream
Golden Spire	Green	Dark green, petioles tinged red	White streaked with pink	Skin golden yellow flesh creamy white
Lord Derby	Green	Dark green, petiole green	Upper side white with tinge of pink, lower side pink	Skin yellow, flesh cream
Seedling 1001 Lord Grosvenor × *Niedzwetzkyana*	Dark purple, wood purple	Reddish purple	Rose purple	Skin purplish red, flesh whitish red tinged red
Seedling 1017	Dark purple, wood purple	Reddish purple (deeper than 1001)	Deep rose purple	Skin yellowish brown, blotches of dull red and crimson, flesh white tinged red

have been used in further breeding work. A description of the seedlings and varieties is given in Table I.

M. Niedzwetzkyana readily crosses with cultivated varieties of apple and produces fertile offspring. It occurs naturally together with *M. malus* in the Caucasus. The principal difference between them is the anthocyanin character previously described, and they are probably only forms of one polymorphic group.

GENETICAL RESULTS

The offspring from the original cross (Lord Grosvenor apple × *M. Niedzwetzkyana*) can be grouped provisionally into two main classes: (1) green, in which the cotyledons and leaves are green, and (2) purple, in which the cotyledons, stems and leaves produce anthocyanin. The plants in the green class are comparatively uniform, but in the purple class the amount of anthocyanin produced varies greatly, varying from only a slight tinge of red to intense pigmentation much deeper than that of *M. Niedzwetzkyana*. There is no difficulty, however, in distinguishing between the two groups. In the earlier families no attempt was made to classify the purple plants in greater detail, but in later families arbitrary classes were made to facilitate description.

The seedlings are first recorded from 6 to 8 days after germination. At this stage the pigment has fully developed. As the seedlings get older the colour tends to diminish, but again increases at the end of the summer. In 1929 two small families 34/29 and 35/29 were raised from inter-

TABLE II

Family no.	Parents	(1) Purple)	(2) Green	χ^2	p
4/20	Lord Grosvenor × *Malus Niedzwetzkyana*	40	39	—	—
34/29	Seedling 1017 × 1001	2	6	—	—
29/32	1001 × 1017	26	12	0·877	0·50–0·30
30/32	1017 × 1001	23	18	7·813	<0·01
9/35	1017 × 1001	54	12	1·636	0·30–0·20
1/36	1017 × 1001	72	22	0·127	0·80–0·70
2/36	1001 × 1017	44	17	0·267	0·70–0·50
Total	1001 × 1017 and reciprocal	221	87	1·731	0·30–0·10
35/29	Cox's Orange Pippin × 1001	3	5	—	—
3/36	Emneth Early × 1017	18	5	5·26	0·05–0·02

crossing the two purple seedlings 1017 and 1001, and from back-crossing seedling 1001 with a green-leaved variety. The results obtained from these F_1 seedlings are given in Table II.

Except for the family 30/32 they conform with a 3 : 1 ratio. The cause of the deviation in this family is not known. These seedlings are, therefore, heterozygous for a single major colour gene. Of two small back-cross families, one (35/29) approximates to a 1 : 1 ratio, but the other (3/36), has a deficiency of green plants.

The varieties Emneth Early and Lord Grosvenor are two of the greenest varieties of apples, and were used in these crosses for that reason. The leaf petioles of some apple varieties are red; as far as possible these varieties have been avoided. Again some varieties produce seedlings which have a tinge of red pigment in the hypocotyl, which

subsequently disappears, comparable with the case in *Raphanus sativus* (Uphof, 1924) and *Rubus idaeus* (Lewis, unpublished), in which this character is due to a single gene. In some families this transitory hypocotyl colour makes the scoring of the purple character a little difficult when the seedlings are very young.

TABLE III

Family no.	Parents	Seeds sown	% of seeds germinated	% of plants surviving
	Green × green; untreated seeds			
14/31	Golden Spire × Brownlee's Russet	73	78·0	65·6
11/31	Cox's Orange Pippin × Laxton's Superb	102	78·4	78·4
16/32	Cox's Orange Pippin × Herring's Pippin	60	96·6	96·6
9/33	Cox's Orange Pippin × Emneth Early	28	71·4	71·4
10/33	Lord Derby × Emneth Early	16	100	100
1/34	Cox's Orange Pippin × Charles Ross	154	64·9	64·9
2/34	Cox's Orange Pippin × Ellison's Orange	126	65·8	65·8
6/34	Ellison's Orange × Cox's Orange Pippin	52	98·0	98·0
3/32	Golden Spire × Beauty of Bath	76	92·1	92·1
	Total	687		
	Mean		78·6	77·1
	Crosses involving *M. Niedzwetzkyana*; untreated seeds			
34/29	1017 × 1001	51	11·7	11·7
35/29	Cox's Orange Pippin × 1001	19	42·1	42·1
29/32	1001 × 1017	73	57·5	56·6
30/32	1017 × 1001	62	72·5	72·5
9/35	1017 × 1001	141	46·8	46·8
1/36	1017 × 1001	127	77·1	77·1
2/36	1001 × 1017	84	72·6	72·6
3/36	Emneth Early × 1017	59	38·9	38·9
	Total	616		
	Mean		56·6	56·4
	Crosses involving *M. Niedzwetzkyana*; treated seeds			
8/37	Cox's Orange Pippin × 1017	52	100	69·2
9/37	Cox's Orange Pippin × 1017	57	98·2	94·7
10/37	Emneth Early × 1017	112	100	82·1
12/37	Golden Spire × 1017	15	100	100
14/37	Lord Grosvenor × 1001	28	100	75·0
15/37	Lord Grosvenor × 1017	20	100	95·0
16/37	1001 × Golden Spire	81	100	82·7
17/37	1001 × 1017	74	100	93·2
18/37	1017 × Golden Spire	67	100	92·5
19/37	1017 × Lord Grosvenor	127	100	83·4
20/37	1017 × 1001	38	100	97·3
	Total	671		
	Mean		99·8	87·7

The percentage of seeds which germinate, under normal conditions, in families derived from *M. Niedzwetzkyana* is usually rather low for diploid apples.

In Table III the percentage of seeds germinated and seedlings potted are given for the three main classes of seeds, which are as follows: Class I: families derived from two green diploid varieties. Class II:

families derived from crosses involving *M. Neidzwetzkyana*, the seeds of which were sown in the ordinary manner. Class III: families derived from *M. Neidzwetzkyana*, the seeds of which were germinated under different conditions, as described later. Considerable variation is found in the percentage germinated in both class I and class II: however, the difference between the means is just on the margin of significance ($t=2{\cdot}32$, $p=0{\cdot}05-0{\cdot}02$).

It was thought at first that the seeds which did not germinate contained deeply pigmented embryos; deeply pigmented seedlings are

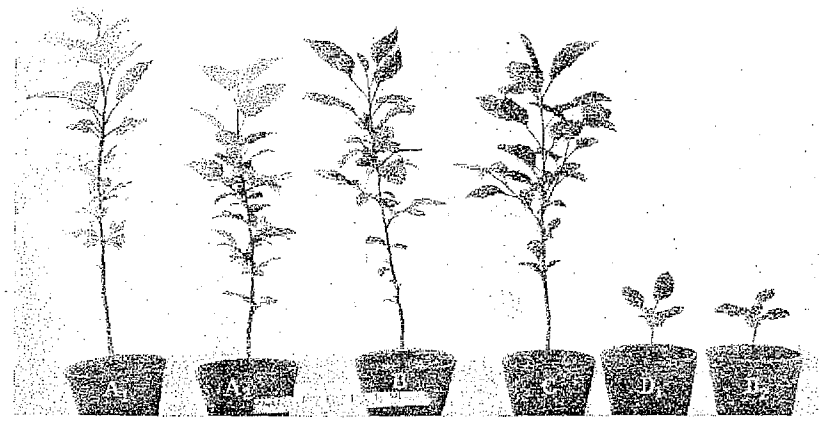

Fig. 1. A_1 and A_2 green, B tinged red, C purplish red, D_1 and D_2 very dark purple plants from family 1/36 (1017 × 1001) photographed 15 months after germination showing the dwarf and stunted growth of the dark purple plants.

always small and stunted, and have up to now failed to make good trees (Fig. 1). It therefore seemed likely that plants which failed to grow satisfactorily would also be weak in their power of germination. Later results showed that this was not the case, and we must conclude that the weakness which accompanies deep pigmentation is not present in the early stages of germination. It is conceivable that the deep pigmentation affects chlorophyll formation or photosynthesis, processes which do not come into play early in germination.

SEED GERMINATION TECHNIQUE

To overcome the difficulty of poor germination a technique for breaking the dormancy of seeds described by Flemion (1933), Tukey

(1934) and R. von Veh (1936) has been applied. They found that by removing the endosperm and nucellar tissue the embryos would start to germinate before the seed was mature. The method adopted during this investigation was to remove the testa, endosperm and nucellar membrane with a sharp scalpel and to place the embryos in sterilized Boveri dishes of wet *Sphagnum* moss. Tap water was found to give satisfactory results; Knop solution did not improve the growth or development of the embryos. The uncovered dishes were placed in a warm greenhouse.

One hundred and fifty embryos were excised on 6 December 1937 (approximately 7 months after flowering). The resulting seedlings grew satisfactorily at first but remained stunted in the spring. The remainder of the embryos were excised 18–22 January 1938. These produced plants which developed normally throughout. This is in agreement with the work of Tukey (1938) who found that if the embryo is immature when excised the resulting seedling is stunted or abnormal.

The development of the embryos after excision is as follows: In twelve hours the cotyledons have divided appreciably; traces of anthocyanin and chlorophyll have appeared on the second or third day. The colour is developed fully in 6–8 days, at which time also the radicle has begun to grow. After 14 days the seedlings which had developed normally were potted up into a standard compost.

In this way 100% of the embryos can be scored for anthocyanin. Approximately 13% of the embryos fail to develop into plants. Some of these defective embryos do not develop beyond the stage of producing pigment and expanding the cotyledons. Others start to produce leaves, but the radicle fails to develop (Fig. 2). These embryos probably carry lethal genes, which affect embryonic development. Not only is the percentage of scorable embryos significantly increased by the treatment from 56·6 to 99·8, but the percentage of embryos which develop into plants is increased from 56·4 to 87·7 (Table III). Therefore only 28% of the seeds which fail to germinate under normal conditions have defective embryos; the remainder (72%) can be induced to grow and form normal plants by removing the testas.

Genetical results from excised embryos

All the seeds obtained from crosses made in 1937 had their testas and nucellar tissue removed. They were scored for anthocyanin 6–8 days later. The purple group was classified in the following way: A, very deep purple; B, reddish purple; C, tinge of red or purple. If these results are compared with those in Table II, it is evident that the proportion of

purple to green plants has not been changed significantly by the treatment. Therefore the seeds which fail to germinate do not contain an abnormal proportion of deeply pigmented embryos, as was suspected. Further evidence for this is obtained from the twenty-six seeds which

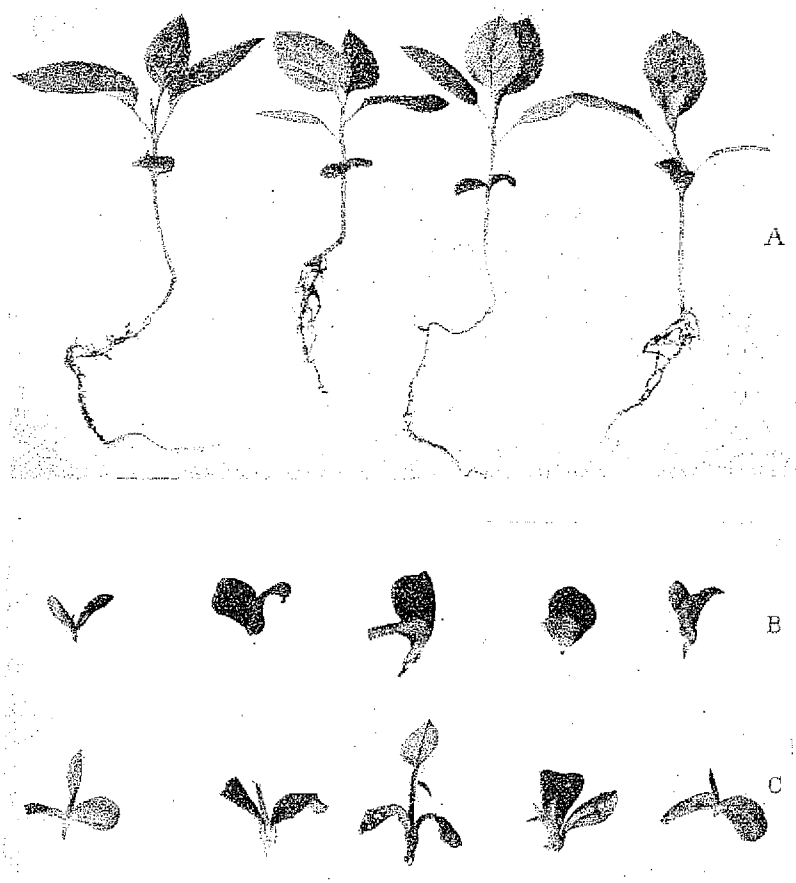

Fig. 2. Seedlings from family 19/37 photographed 20. i. 38, 45 days after excision of the embryos. *A*, normal seedlings. *B*, seedlings which fail to develop either leaves or roots. *C*, seedlings which develop leaves but the roots fail to develop. $A = \frac{3}{4}$, *B* and $C = \frac{3}{2}$ natural size.

did not develop beyond opening the cotyledons in families 18/37 and 19/37; twelve were purple and fourteen green, a close approximation to the expected 1 : 1 ratio.

When the two F_1 seedlings (1017 and 1001) are intercrossed the results approximate to a 3 : 1 ratio, and back-crossing either seedling to

a green variety approximates to a 1 : 1 ratio. The original scoring of this family was fifty-six purple and twenty-five green, but as the plants developed it was evident that eight plants grouped in the C class were really greens. The deficiency of greens in family 16/37 is probably due to the green parent carrying one or more factors for coloured hypocotyl. This obscures the scoring, so that some plants which are really green are placed in class C.

TABLE IV

Family no.	Parents	Purple A	B	C	Sum ABC	Green D	χ^2	p
9/37	Cox's Orange Pippin × 1017	17	11	14	42	48	0·400	0·70–0·50
10/37	Emneth Early × 1017	12	22	19	53	54	0·009	0·95–0·90
12/37	Golden Spire × 1017	0	0	3	3	12	5·400	0·05–0·02
15/37	Lord Grosvenor × 1017	6	3	3	12	8	0·800	0·50–0·30
18/37	1017 × Golden Spire	—	—	—	48	38	1·152	0·30–0·20
19/37	1017 × Lord Grosvenor	—	—	—	65	81	1·753	0·20–0·10
					223	241	0·698	0·50–0·30
11/37	Golden Spire × 1001	0	0	0	0	2		
13/37	Lord Derby × 1001	0	1	0	1	1		
14/37	Lord Grosvenor × 1001	5	3	4	12	15	0·333	0·70–0·50
16/37	1001 × Golden Spire	9	19	20	48	33	2·776	0·10–0·05
		14	23	24	61	51	0·892	0·50–0·30
17/37	1001 × 1017	9	19	23	51	21	0·666	0·50–0·30
20/37	1017 × 1001	10	8	11	29	8	0·225	0·70–0·50
		19	27	34	80	29	0·149	0·70–0·50

Discussion

The equality of green and purple plants obtained when Lord Grosvenor (green) was crossed with *Malus Neidzwetzkyana* (purple) indicates that the latter is heterozygous for a single major colour gene. The variation in the depth of pigmentation found in the purple classes in all families can be explained by either a difference in dosage of the same gene or by the effect of modifiers on the principal colour gene.

The fact that two seedlings, 1017 and 1001, behave genetically in the same way, although one is much more deeply pigmented than the other, indicates that the difference between these two plants is not due to different dosages of the same gene. Furthermore, seedlings are obtained from a back-cross of the heterozygote to the green which are as deeply pigmented as the darkest plants produced by intercrossing two heterozygotes.

Further support of the hypothesis that modifiers are responsible for variation within the purple class, is (1) that a close approximation to 3 : 1 and 1 : 1 ratios is obtained from intercrossing and back-crossing

respectively, and the same range of variation occurs in both intercross and back-cross families; and (2) the inheritance of the pigmented character is the same in seedlings 1017 and 1001, although they are phenotypically different. It is also known that cultivated apples are invariably heterozygous, a condition favoured by the prevalence of self-sterility and maintained by clonal reproduction. This would favour the segregation of modifying genes, thus accounting for the variation in the expression of the colour gene.

A further generation of plants would have to be raised, to determine whether duplex plants are present among the F_2. It is, however, evident that even if the major gene is in a polysomic condition modifiers play an important role in the activity of the purple gene.

Haldane (1933) has pointed out that in a polyploid most of the genes will eventually return to the diploid condition and, therefore, disomic segregation of the purple gene would not be in disagreement with the secondary polyploid theory.

SUMMARY

The inheritance of a "purple anthocyanin" character in apples is described. *Malus Neidzwetzkyana* was found to differ from *M. Malus* in having a single dominant gene for "purple pigmentation". Great variation was found within the "purple" plants, probably due to the segregation of modifiers.

A technique for increasing the percentage of germination by removing the testa is described. The percentage of scorable plants was increased from 56 to 99. No differential viability of the "green" and "purple" embryos was found.

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