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THE GENETICS AND CYTOLOGY OF DAHLIA SPECIES.

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(With One Coloured Plate and Five Text-figures.)

CONTENTS.

INTRODUCTION.

THESE experiments were begun as an attempt to elucidate by genetic means certain peculiar distributions of flower colour evident in the garden dahlia. Investigations relating to this particular part of the work are still in progress. The following account is preliminary to the more extended series of observations it is hoped to present later.

MATERIAL AND METHODS.

The garden dahlia can be grown from seed to maturity in one year. Suitable treatment brings the plants into flower in July, the flowering period continuing up to the time of the autumn frosts. The "tubers," if lifted and stored during the winter, can be grown on from year to year indefinitely.

The Compositae present material which does not readily lend itself to genetical analysis, and with *D. variabilis* this only becomes practicable to any extent by reason of the general self-sterility which prevails.

Cross-pollination was made in these experiments by gently brushing the disc of the capitulum used as a male over the disc of the female. Capitula selected for crossing were bagged before the disc florets opened, and a plug of cotton wool was inserted in the mouth to secure better protection from insects—especially earwigs. Seed ripens in 7–8 weeks.

All the usual precautions against contamination by foreign pollen have been observed throughout these experiments.

THE GENUS *DAHLIA*.

From inspection of the botanical descriptions of *Dahlia* species it is evident that, with the exception of *D. variabilis*, they can be assigned to one of two groups for flower colour: Group I (ivory-magenta-purple); or Group II (yellow-orange-scarlet).

GROUP I (ivory-magenta-purple).

*D. Merckii**	*D. Maximiliana*	*D. pubescens*
*D. Maxoni**	*D. imperialis**	*D. platylepis*
D. excelsa	*D. dissecta*	*D. scapigera*
D. Lehmanni		

* I have grown and observed these species.

D. Merckii Lehmann. 3 ft. high. Ray florets ♀ and fertile, pale violet to purplish violet. Disc florets purplish. First leaves simple.

D. Maxoni is described by Popenoe (1920) as growing 15–18 ft. high, and, in the wild form, bearing lilac-pink or white rays. Under cultivation other varieties occur—"almost a pure pink, a deep lilac pink and a darker shade which could almost be called a mauve." First leaves ternate-pinnate.

D. excelsa Benth. is said to have pale rose-purple or purple ray florets. Loudon's (1843) figure of this species seems identical with the photograph of *Maxoni* in the *Am. Journ. of Heredity*. Grows 15–30 ft. high.

D. Lehmanni Hieron. 12 ft. high with pale lilac rays.

D. Maximiliana Hort. Ray florets a delicate mauve. The plants of *Maxoni* which I have grown are very near to, if not identical with *Maximiliana*. The four dahlias *Maxoni, excelsa, Lehmanni* and *Maximiliana* may be varieties of one and the same species. The recorded differences between them are no greater than similar variations observed *within* other species.

D. imperialis Roezl. 6–18 ft. high. Ray florets white, very faintly flushed lilac-pink, with a crimson spot at base of petals.

D. dissecta S. Wats. Deep purple rays, 1½–2 ft.

D. pubescens A. Brougn. *ex* Newman Pale purple or mauve rays; 1½–2 ft.

D. platylepis A. Brougn. *ex* Newman. Lilac rays; 1½–2 ft.

D. scapigera Knowles and Westcott, is described by Bentham and Loudon as having pure white ray florets, but Loudon's figure of this species shows faintly tinged violet rays; 1–2 ft.

GROUP II (yellow-orange-scarlet).

*D. coccinea** *D. coronata** *D. gracilis* *D. tenuis*

* I have grown and observed these species.

D. coccinea Cav. 5–9 ft. high. Ray florets yellow, orange or scarlet-orange. I have not observed the yellow variety, but it has been recorded by Loudon and others. First leaves simple. Petals stellate or imbricate.

D. coronata Hort. 4–5 ft. high, bearing scarlet-orange to scarlet rays. Sweet scented. Petals incurved. First leaves simple.

D. gracilis Ortgies. Ray florets scarlet-orange.

D. tenuis Robinson and Green. 15 in. high, ray florets yellow.

The above list of *Dahlia* species is by no means complete, since others have been collected. Few of these latter have been grown under cultivation or adequately described, and apparently considerable confusion exists as to their identity.

DAHLIA VARIABILIS DESF.

(1) MORPHOLOGICAL OBSERVATIONS.

The position of *D. variabilis* with regard to the colour groups is unique in that it unites both series within itself. Whites, ivories, yellows, scarlets and purples, with many intermediate colours and intensities, can be seen in various patterns combined with various flower shapes.

These flower colours and their distribution in the garden dahlia are the result of the presence or absence of two series of soluble pigments: (1) flavones, (2) anthocyanins.

The flavone series is expressed in an almost continuous gradation of colours ranging from ivory to deep yellow (Plate XII, figs. 2–6), all of which give the characteristic flavone reaction when fumed with ammonia, *i.e.* the ivory flowers turn a bright lemon yellow; the yellow change to an intense orange. Flowers intermediate between ivory and yellow change, when fumed, to shades intermediate between yellow and orange.

Among the flowers which, upon visual examination alone, might easily be classed as ivories, certain kinds are found which give no reaction with ammonia. In this account such forms are called "whites" as distinct from ivories which, though visually similar, always give a positive reaction for the presence of flavone.

The anthocyanin colours may be arbitrarily classified in three groups: (*a*) magenta to deep purple, (*b*) pale orange to deep scarlet, (*c*) colours intermediate between (*a*) and (*b*).

Given a wide enough range of present-day varieties for inspection it quickly becomes evident that all the colours of the magenta-purple group are invariably associated with ivory flavone grounds; and that all the colours of the orange-scarlet group are invariably associated with yellow flavone grounds. (It will be noticed that this association corresponds with that of the two colour groups previously referred to in which *Dahlia* species may be classified.) The intermediate flower colours are associated with intermediate flavone grounds.

Visual examination of the flower colours as to their kind, association and constitution is much facilitated by the occurrence of (*a*) mosaic flowers in which the anthocyanin is distributed in flecks and streaks, (*b*) patterns where the anthocyanin covers a portion only of the petal, and (*c*) relatively frequent somatic mutation of both anthocyanin and flavone pigments.

In mosaic flowers the distribution of anthocyanin is sharply discontinuous, forming flecks and streaks—the flavone ground colours showing where no anthocyanin occurs. Mosaic flowers in the magenta-purple colour group always have ivory grounds; in the orange-scarlet group they have yellow grounds. Another type of colour distribution is that in which the anthocyanin occurs as a flush across the middle of the petals exposing the flavone ground at the tips and bases, and again the same association of flavone and anthocyanin colours is to be seen. Further evidence as to this relation is afforded by somatic mutation involving each series of pigments.

(a) Somatic mutations of anthocyanins and flavones.

(i) *Anthocyanins.* Apart from mosaicism, plants with coloured flowers occasionally sport sectors without anthocyanin—usually quite narrow, rarely so much as $\frac{1}{4}$ in. wide, and running the length of the petal. Orange and scarlet flowers have yellow sectors; magenta-purple flowers, ivory sectors (Plate XII, figs. 10, 11).

Not uncommonly dilution or intensification of the anthocyanin occurs. All of the pigment may be missing from a sector, and immediately adjacent on one side another sector, usually corresponding in size with the uncoloured portion, will have a double dose of anthocyanin (Plate XII, fig. 11).

In such a case it is probable that a mitosis has occurred such that one of the daughter cells has received all of the chromosomes or factors for colour—the other receiving none—and that these cells have by division given rise to the uncoloured and doubly-coloured segments respectively. Attention is drawn to the similarity of this type of somatic mutation to that observed by Eyster (1924) in variegated maize cobs[1].

A similar condition may occur without the entire loss of anthocyanin, dilute and deeper stripes being found side by side (Plate XII, fig. 12). More rarely a single, solitary deep or dilute stripe occurs unaccompanied by any other visible change.

Petals have been seen where several of these mutations have been present side by side.

(ii) *Flavones.* Yellow dahlias have been observed to sport ivory and white sectors. Similarly, ivory flowers have sported white sectors. In the ivory sporting to white, it is much more difficult to recognise where a change has occurred. In all these experiments the final criterion of the identity of a white or ivory flower has been the fuming of the petal with ammonia. With practice however it is possible to distinguish white from ivory by eye—the white having a peculiar translucent appearance and seeming to be very faintly tinged pink or mauve. This is due to the reflection of light and is merely an optical effect.

The question arises as to what results if the yellow and ivory flavones sport in the presence of anthocyanin. Variations of this kind have been observed—clearly showing the association of particular flavones with particular colours.

[1] Miss de Winton who is working on *Primula sinensis* at this Institution tells me she has observed similar variations in flower colour, and that they always occur in plants heterozygous for the factor concerned. (See also footnote to p. 138 of this paper.)

(*a*) If the yellow flavone ground of a crimson-scarlet flower sports to ivory, then the flower colour will be *purple*, not scarlet (Plate XII, fig. 14).

(*b*) If either yellow or ivory sports to white, then *no* anthocyanin develops.

Confirmation of (*a*) is provided by flowers which have yellow flavone in the basal half of their petals, shading off almost to ivory at the tips. In these flowers it would be expected that anthocyanin on the basal half would appear scarlet shading off to purple at the tips, and such is in fact the case (Plate XII, fig. 8). Moreover the mosaic variety "Dorothy" has a yellowish ground with crimson flecks, which frequently sports an ivory ground. On the ivory ground the anthocyanin appears purplish (Plate XII, fig. 13).

That it is the flavone which changes and not the anthocyanin alone in the cases just mentioned is made still clearer by bleaching the anthocyanin in an aqueous solution of SO_2, which does not affect the flavones. *In every case the magenta or purple sectors have ivory grounds—not yellow.*

Two such plants have been used in breeding and they have proved to be heterozygous for yellow, and have given magenta or purple seedlings in F_1.

The position with regard to the loss of ivory flavone from a flower carrying yellow flavone and anthocyanin is not clear. Although it is difficult to perceive ivory in the presence of yellow flavone, yet, as will be shown, presence or absence of the ivory pigment does make a proportionate difference to flavone colour. It is to be expected therefore that, should ivory be lost when yellow flavone and anthocyanin are present, a change will occur in the colour of the flower. Sectorial variations have been observed which have every appearance of being due to such a change.

In the mutations so far described apparently only the colour of the flower changes, and not the intensity of pigmentation. Thus an orange flower sports pale magenta sectors; deep orange, deeper magenta; scarlet, purple—and so on.

Intermediate Forms. The facts so far presented have been from the two extreme groups: (1) ivory-magenta-purple, (2) yellow-orange-scarlet. Mention was made of intermediate colour forms, which are more difficult to understand. Careful examination of many hundreds of these has led to the establishment of the following facts:

(1) The yellow and ivory flavones may be present together in varying amounts.

(2) Yellow flavone partially obscures ivory.

(3) The proportion of ivory to yellow gives rise to several shades of colour ranging from ivory, through cream and primrose, to yellow.

(4) When anthocyanin is present the flower colour is determined by the proportion of ivory and yellow flavones, *e.g.* on an ivory ground it is purple; on cream, more crimson; on pale yellow, nearer scarlet; on a good yellow ground, scarlet. The precise limits of the range of the flower colour as determined by the association of anthocyanin and flavone are not clear at present, but so far as my observations have gone the above scheme seems to be approximately correct, though doubtless many moderating influences of a minor nature frequently come into play.

(*b*) *Distribution of anthocyanins and flavones.*

(i) *Anthocyanins.* As previously stated anthocyanin is developed only when flavone is present. It is obvious therefore that where anthocyanin is present the flower colour is due to the combined effect of the anthocyanin and flavone, and that only chemical analysis can finally establish the true colour of the particular anthocyanin(s) involved. Any reference here to an anthocyanin colour by name is made with this reservation.

Apart from evidence to be quoted later there is some reason to believe that at least two distinct anthocyanins occur in the garden dahlia, namely (1) a geranium red, (2) purple. Each is found in several intensities (the above names merely indicating certain degrees of pigmentation), but their distribution and qualitative variation and their reactions with ammonia and sulphurous acid differ appreciably.

Since the geranium red anthocyanin is never present alone, but associated with flavone, the colour of the flower as seen may generally be described as "scarlet."

The "*scarlet*" is the less variable of the two anthocyanin colours. The tinged, pale orange, deep orange, scarlet and deep scarlet colours may be different concentrations of the same pigment, for it is possible to make an almost perfect gradation from the most faintly tinged to the deepest of scarlets. Especially is this true if flowers of different ages are used, since there is a considerable fading with age of both anthocyanins and flavones, distinct colour varieties overlapping each other. Thus the intensity of colour of a fully expanded flower of variety "A" will be identical with a newly opened flower of variety "B," and so on.

This fading of flower colours begins with the unfolding of the petals

and suggests that the production of anthocyanin is complete (or practically so) at the opening of the capitulum, and that increase in size of the petals brings about a corresponding dilution of the pigment, the colour differences between some varieties being merely quantitative.

One exception has been observed. A certain pale magenta flowered variety is not fully coloured until the flower is expanded—the buds and the young flowers being almost pure ivory.

The distribution of anthocyanin may vary, the concentration being least at the tip of a petal; least at, or even absent from, both tip and base; deeper on the backs of the petals or *vice versa*; concentrated on the edges or in the middle of the petals, etc.

Different individuals with apparently the same yellow or ivory grounds may have different intensities of anthocyanin, from the faintest tingeing to very intense coloration—as if varying doses of the same pigment occurred on the same ground.

The quantitative variations described for "scarlet" anthocyanin were observed in flowers whose yellow grounds were apparently identical.

In the scarlet series the presence of small amounts of ivory flavone may alter qualitatively the observed colour of the flowers, but in the purple series no such interference is possible. Hence any qualitative variation in this series is likely to be (1) the direct expression of the modification of anthocyanin or (2) due to the occurrence of different anthocyanins.

Variations may be observed in the magenta-purple series (*a*) inclining to rose, (*b*) inclining to violet, and there are cases in which they seem to occur together in the same flower.

It is interesting to note that apparently a similar variation occurs in *D. Maxoni*.

Pigmentation of the stems of the dahlia, though distinct from flower colour, is nevertheless not entirely unrelated. White and ivory varieties never have colour in the stems or in the sub-epidermis of the tuberous roots—yellows rarely have. The yellows which have faintly tinged stems are not at present understood, and seem to be exceptional. All other coloured varieties observed have coloured stems.

There are several well-known varieties which have deeply coloured leaves and stems, and in such plants this pigmentation extends also to the disc—florets, bracteoles, etc. all being coloured. Various intergrades have been seen between the deepest and the palest of these deeply pigmented plants. Presence of abundant stem pigment confers an added intensity upon the flower colour.

Normally, all variations of flower colour are only expressed in the ray petals—the disc florets remaining the usual yellow colour—*i.e.* flavones are present and give the characteristic reaction with ammonia in every part of the capitulum except the pollen. Thus the disc florets of a normal white rayed variety all carry flavones—the petals alone having no pigment.

There is another kind of white, however, in which pigments are absent from the whole of the capitulum—bracts, bracteoles, disc florets and rays. This white is the extreme expression of a condition which most frequently shows itself in coloured edges to otherwise white petals. The well-known variety "Union Jack" is typical of this condition. I have attempted to analyse the behaviour and inheritance of this abnormal pigment distribution, but it appears to be extraneous to the general distribution and inheritance of pigments in *Dahlia*. The results of this part of the investigation will appear in a later paper, when the work has been carried further.

(ii) *Flavones.* Different intensities of yellow flavone may be present in a petal, all the petals of a given plant showing the same distribution. A common distribution is one in which the basal quarter, third, half or two-thirds of the petal is cream, primrose or yellow, passing to ivory, cream or primrose at the tip. Anthocyanin on such a ground appears mostly scarlet at the base and bluish toward the tip (Plate XII, figs. 7 and 8).

(c) *Reactions of anthocyanins and flavones with ammonia and SO_2.*

As indicated on p. 128 the change of colour occurring when white, ivory or yellow petals are fumed with ammonia is as follows:

Petal colour	Changes to
White	No change
Ivory	Lemon yellow
Yellow	Intense orange

The ammonia test is a well-known method used for detecting the presence of anthocyanin in plant tissues. If anthocyanin is present a green or bluish colour usually develops upon fuming, though possibly other substances in the cell sap may modify this green or bluish appearance. In addition to the many fumings made with ammonia in the course of these experiments, petals have been bleached in SO_2, and the reactions of different flower colours with these two reagents noted.

The observations may be briefly summarised as follows:

SO_2. SO_2 does not affect the flavones. It half bleaches the deeper

coloured petals and almost entirely bleaches tinged varieties. Penetration is increased if the petal is first fumed in ammonia and then bleached —complete removal of the anthocyanin pigments resulting, thus revealing the flavone ground.

Ammonia. (*a*) Magenta and purple flowers give green and bluish-green reactions. (*b*) Orange and scarlet give an intense reddish-brown coloration. (*c*) Intermediate forms give intermediate reactions.

The intense reddish-brown which develops when orange or scarlet petals are fumed is of some interest, since this is not the typical reaction of anthocyanin with ammonia[1].

All the other species I have grown conform, after their kind, to the above results.

(2) Breeding results.

(*a*) *Flavones.*

The genetical analysis of a Composite like *Dahlia* would be hardly possible but for the self-incompatibility which prevails.

A typical "single flowered" variety has 8 ray petals and about 130 ♀ disc florets which open successively from the periphery of the disc inwards. The ray florets of all varieties I have seen are sexually non-functional, and at the most bear only rudimentary ♀ sexual organs. Norton (1926), however, says some varieties produce seed in the ray flowers.

The filaments of the stamens are elastic and allow the anthers and their 5-valved membraneous covering to be extruded above the corolla tube by the growth of the style within. Dehiscence is accompanied by the liberation of the stigmas through the summit of the membrane and withdrawal of the stamens—the stigmas emerging covered with abundant pollen. In fine weather the stigmas are probably receptive a few hours later.

The smallness of the floret and early dehiscence of the anthers make emasculation impracticable.

Each flower head is potentially able to set about 130 seeds, but for various reasons more than 90 seeds rarely develop under natural conditions. As far as possible, cross-pollinations have been made when all the stigmas of the disc florets were expanded. The inclemency of the

[1] Since going to press I have seen the paper by Buxton and Darbishire "On the Behaviour of 'Anthocyanins' at varying Hydrogen-ion Concentrations" (*Journ. Gen.* xxi. 1). It is interesting to note that these authors, in their experiments on anthocyanin colours of various flowers, found them to fall into two distinct, main groups with which the reactions of the two Dahlia anthocyanins closely correspond.

weather has often prevented the choice of this ideal time for pollination, hence the smaller numbers of seeds obtained in certain crosses.

Self- and cross-incompatibility. Detailed records have been kept of the self-pollination of 48 distinct varieties. 217 capitula have been selfed—the equivalent of over 28,000 florets—giving 132 seeds or 0·47 per cent. Of these 132 seeds 95 germinated. The average number of selfed seedlings reaching maturity is only 0·25 per cent. of the flowers pollinated. Norton (1926) also records that from about 30 varieties selfed he only obtained seed from one.

The plants raised from selfing were usually smaller and less vigorous than the average. Two which were identical in every respect with their parents were undoubtedly apogamous seedlings. I have not yet seen any two plants in the families I have raised which were not to be distinguished one from the other.

Of the plants obtained from selfing, 60 were from 3 individuals. These gave (1) 20 plants from the selfing of more than 27 capitula, (2) 29 plants from 43 capitula and (3) 8 plants from 20 capitula respectively. The reason for the increase in self-fertility in these three instances is not clear, and seems to be unusual—most of the plants tested never giving rise to a single seed. In addition to the recorded selfings several other varieties have been selfed, no seed setting whatever.

For all practical purposes the occasions when a plant's pollen will compete successfully with that introduced in controlled cross-pollination are so rare that they may be ignored. No such instance has been detected in these experiments.

Since both pollen and ovules—or at least a high proportion of them—are functional, it is evident that the failure to obtain seed from self-pollination is due to self-incompatibility. Cross-incompatibility frequently occurs, and sometimes constitutes a handicap to close breeding. An attempt was made to obtain information regarding self- and cross-incompatibility but was abandoned because of the complexity of the results, *e.g.* selfed seedlings have been compatible as males on their mother but have failed when the reciprocal cross was made. The incompatibility [illegible] complete or partial—"one way" incompatibility often occurring—the latter phenomenon doubtless due to polyploidy.

Flavone inheritance. The flavones seemed the easiest characters to work with although no simple solution of their inheritance was expected, since, as I have shown later in this paper, cytological examination proves the garden dahlia to be an octoploid.

The data to be presented will show that the inheritance of flavones is

governed by two independent factors—*Y* for yellow and *I* for ivory flavone, so that *YI* and *Yi* plants have yellow, *yI* ivory and *yi* white flowers.

Y is carried by four homologous chromosomes, which, assorting at random during meiosis, give tetrasomic segregation of *Y*.

I is also tentatively assumed to be carried by four chromosomes for reasons to be stated later, but, so far, disomic segregation only has occurred for this factor.

Table I shows the F_1's from crosses between whites, ivories and yellows.

TABLE I.

Fam.	♀	♂	Seeds	Germinated Plants	F_1 Yellow	F_1 Ivory	F_1 White	Expectation from tetrasomic segregation of *Y* and disomic segregation of *I* Yellow	Ivory	White
9/28	35/26 (white) *yyyyii*	32/26 (white) *yyyyii*	44	37	35	—	5*	30	—	—	35
10/28 and 12/28	32/26 (white) *yyyyii*	White Star (ivory) *yyyyIi*	35	31	31	—	16	15	—	15·5	15·5
40/28	35/26 (white) *yyyyii*	22^9/27 (yellow) *YYyyIi*	55	52	50	41	5	4	41·6	4·16	4·16
41/28	32/26 (white) *yyyyii*	31^5/27 (yellow) *YyyyIi*	54	38	36†	15	9	11	17·5	8·75	8·75
6/28	14/26 (yellow) *YYYyIi*	Ideal (yellow) *Yyyyii*	52	51	51‡	49	0	0	49	0	0

* See text. † 1 plant unrecorded for colour. ‡ 2 plants unrecorded for colour.

From Families 10 and 12/28 it is obvious that the difference between ivory and white, in this instance, is the presence and absence of a single factor, *I*, for ivory.

On this assumption there should have been no ivories in family 9/28, and the discrepancy remained unexplained until it was discovered that the parent 35/26 was a mosaic of ivory and white. Numerous flowers of this plant have since been tested and the large majority found to be white. Occasionally ivory-rayed capitula have been found—but when ivory occurs it is usually as a portion of a capitulum or petal. In the next cross it will be noticed that the capitulum of 35/26 used was, genetically, pure white.

In family 40/28 the ratio of 41 yellows : 5 ivories : 4 whites is a close

approximation to the segregation expected from random assortment when parents of the constitution *iiyyyy* and *IiYYyy* are used. An attempt was made to identify differences between the 41 yellows, but although there were obviously slight differences in shade, the similarity was too great to allow of further classification. Of the five ivories one was doubtful, the reaction with ammonia being weak and patchy (possibly the result of mosaicism?).

The germination of seed in family 41/28 is poorer because the "abnormal white" condition involved in this and certain other crosses (but not included in the figures) is usually associated with lessened viability. Nevertheless the deviation from the expected ratio is small. The yellow parent may be assigned the factorial constitution *IiYyyy*.

The next family (6/28) was all yellow—but showed 3 distinct intensities with minor differences of distribution and shade. The three groups consisted of 21 plants practically identical in colour with their mother, 13 paler and 14 deeper (+ 1 unclassified). Several instances of somatic mutation were noticed and tested—5 plants showing white and 2 ivory sectors. The male parent "Ideal" often shows small uncoloured sectors. It is not quite certain whether these are white or ivory (a fairly large sector is required for a satisfactory test with ammonia), but it is probable that "Ideal" sports white sectors. The ♀ has never sported.

As shown later (Fam. 32/27) "Ideal" is probably *Yyyyii*. Thus the individuals revealing a white ground when *Y* is absent in Fam. 6/28 can only be derived from a plant (*i.e.* 14/26) producing *i* gametes. It follows therefore that 14/26 is probably heterozygous for *I*.

In Table II are shown various crosses made with 14/26. The other parents carried anthocyanin which is for the present ignored, the flavone grounds only being considered. "Union Jack," 34/26 and M 5 are all

TABLE II.

Fam.	♀	♂	Seeds	Germinated	Plants	Yellow ground	Ivory ground
23/27 and 24/27	14/26 (yellow) *YYYy*	Union Jack (yellow ground) *Yyyy*	146	126	126	125	1?
38/28 and 39/28	Union Jack (yellow ground) *Yyyy*	14/26 (yellow) *YYYy*	64	50	49	49	0
4/28 and 5/28	14/26 (yellow) *YYYy*	36/26 (ivory ground) *yyyyII*	109	81	79	78	1
15/26	14/26 (yellow) *YYYy*	M 5 (yellow ground) ?	57	57	57	57	0
					Total	309	1 + ? 1

known to produce gametes recessive for *Y*, while 36/26 has an ivory ground. Hence if 14/26 produced non-yellow gametes, ivories or whites should appear in F_1. Out of 311 plants only 1 had an ivory ground. Another was scored earlier in these experiments as "? purplish-magenta," but almost certainly had not an ivory ground.

These results seem to indicate that 14/26 is triplex[1] (*YYYy*) or quadriplex (*YYYY*) for *Y*, producing nothing but yellow grounds when crossed with other plants. In family 5/28, however, an ivory-ground individual appeared in the F_1 of the cross (14/26 *YYY*(*Y*)*y* × 36/26 *yyyy*). If we assume 14/16 to be triplex for *Y* the unexpected ivory plant could then have arisen by (1) non-disjunction, ultimately giving rise to a zygote deficient in the *Yy* chromosome set, or (2) irregularity of disjunction in the equational division, bringing about *YY* and *yy* instead of *Yy* gametes[2].

Unfortunately a chromosome count was not made of this unexpected recessive individual. Similar exceptional recessives, however, are being examined.

Blakeslee, Belling and Farnham (1923) encountered precisely the same irregularity in their work on the tetraploid Daturas. The chromosome number of two of the unexpected recessive individuals was normal. The exceptional recessives form slightly over 2 per cent. of the offspring when the type *AAAa* is back-crossed to *aaaa*, but when the same triplex variety is crossed to *Aaaa* only about 1 per cent. of exceptions are produced—a difference to be expected as *Aaaa* parents have only half the number of gametes of the formula *aa*.

Table III shows the results of further crosses in which the parents had ivory or yellow grounds. In families 8/28 and 11/28, 16/28 and 17/28 the female parent in each case is probably homozygous for *I* since no whites appear when "White Star" (*Ii*) is used as a male. M 2 (family 17/28) is evidently simplex (*Yyyy*) for *Y*.

[1] Following Blakeslee, Belling and Farnham (1923) the terms quadriplex (*YYYY*), triplex (*YYYy*), duplex (*YYyy*), simplex (*Yyyy*) and nulliplex (*yyyy*) are used to denote the genetic constitution of plants carrying flavone and anthocyanin factors.

[2] With reference to the inference that 14/26 is triplex for *Y* it is probably significant that in family 5/28 certain plants which had pale orange flowers with yellow grounds were observed to sport magenta sectors (*i.e.* the yellow ground changed to ivory). If this sporting be due to the loss of a *Y* factor or chromosome it would only become apparent in individuals simplex for *Y*, and such could only have appeared in this family if 14/26 produced *Yy* gametes.

Mutations similar to the above have been observed in as many as seven different families, but in every case they have arisen only in families which are known to include forms simplex for *Y*.

TABLE III.

Fam.	♀	♂	Seeds	Germinated Plants	F_2 Yellow grounds	F_2 Ivory grounds	Expectation Yellow grounds	Expectation Ivory grounds
8/28 and 11/28	36/26 (ivory ground) *yyyyII*	White Star (ivory) *yyyyIi*	49	39 39	0	39	0	39
16/28	M 8 (ivory ground) *yyyyII*	White Star (ivory) *yyyyIi*	38	33 27	0	27	0	27
17/28	M 2 (yellow ground) *YyyyII*	White Star (ivory) *yyyyIi*	48	43 43	21	22	21·5	21·5
32/27	Union Jack (yellow ground) *Yyyy*	Ideal (yellow) *Yyyyii*	11	10 8	7	1	6	2
30/27	Union Jack (yellow ground) *Yyyy*	32/26 (white) *yyyyii*	11	9 7	4	3	3·5	3·5

The numbers of family 32/27 in Table III, though small, are of some value in that, apart from irregularities, the occurrence of an ivory individual in family 32/27 implies the production of non-yellow gametes by "Ideal," which must be duplex or simplex for *Y*. This is in accordance with expectation, for the white sectors which "Ideal" sports are probably due to the loss of a *Y* factor in the soma of a plant which is simplex for *Y*, thereby revealing the white ground.

Before proceeding to the survey of other details relating to the cross-pollination of dahlias for flavone inheritance, two F_1's from selfing must be mentioned.

The first of these was from a natural seedling of "Union Jack"—27/24. From the selfing of more than 27 capitula 20 plants were raised. 27/24 is a purplish variety and all of the F_1 plants were magenta or purplish, *i.e.* the plant probably breeds true for ivory ground.

In the second instance from the selfing of 43 capitula of "Union Jack" 29 plants were raised. 21 of these had yellow grounds, the remaining 8 ivory—approximately 3 yellows to 1 ivory. An individual of the constitution *Yyyy* selfed would give this result.

Turning now to Table IV we may see how far the cross-pollinations directly or indirectly involving "Union Jack" and 27/24 agree with expectation.

Seedlings 1^1/25 and 1^2/25 were from "Union Jack" selfed. 1^1/25 has an ivory ground and crossed with 1^2/25 gave approximately 5 yellow grounds : 1 ivory. This indicates that 1^2/25 is duplex for *Y* and should give an 11 : 1 ratio of yellow to ivory grounds when back-crossed to

TABLE IV.

Fam.	♀	♂	Seeds	Germinated	Plants	F_3 Yellow grounds	F_3 Ivory grounds	Expectation Yellow grounds	Expectation Ivory grounds
34/27, 35/27, 29/27 and 56/28	1²/25 (yellow ground) *YYyy*	Union Jack (yellow ground) *Yyyy*	190	151	148	134	14	135·67	12·3̇
36/27	1²/25 (yellow ground) *YYyy*	1¹/25 (ivory ground) *yyyy*	29	21	20	16	4	16·7	3·3
37/27	1²/25 (yellow ground) *YYyy*	2⁷/24 (ivory ground) *yyyyII*	80	66	52	49	3	43·33	8·6
22/27	Glenshee* (yellow ground) *YYyy*	Union Jack (yellow ground) *Yyyy*	90	78	76†	64	12	63·34 (on a 5 : 1 ratio see text)	12·6̇
26/27 and 31/27	Union Jack (yellow ground) *Yyyy*	34/26 (yellow ground) *YYyy*	99	75	74	66	8	67·84	6·16

* Glenshee selfed gave one pale magenta with an ivory ground.
† 2 plants destroyed.

"Union Jack." The actual figures agree almost perfectly. In family 36/27 the expected numbers are closely approached, as also in the cross "Union Jack" × 34/26 if we assume that 34/26 is duplex for *Y*.

The two remaining families show unexpected results.

The appearance of ivories in family 22/27 shows that "Glenshee" must be duplex or simplex for *Y*. The F_1, however, gives a perfect 5 : 1 ratio, a result which would be obtained if a plant duplex for *Y* were crossed with the quadruple recessive *yyyy*.

East (1925) and his co-workers discovered sterility[1] factors in *Nicotiana Sanderae* of such a kind that a plant carrying a factor S_1 inhibited the growth of all S_1 pollen in its styles. Thus in a cross $S_1S_2 \times S_2S_3$ only S_3 pollen could penetrate the stylar tissue of the female to unite with S_1 or S_2 ovules. In 1926 Brieger and Mangelsdorf reported linkage between a colour factor *C* and the sterility factor S_2. In certain crosses involving the linked factors only the non-coloured pollen —apart from crossing over—was effective—*e.g.* the cross $S_1cS_2c \times S_3cS_2C$ gave in F_1 75 per cent. of whites instead of equal numbers of coloured and whites.

[1] Or, more correctly, "incompatibility" factors.

"Glenshee" and "Union Jack" are one-way incompatibles; "Glenshee" always setting abundant seed when crossed by "Union Jack," but the reciprocal cross rarely producing any seed. Hence adopting East's interpretation of incompatibility these two varieties must carry a sterility factor (*S*) common to both. If we assume that the factor *Y* is linked with a sterility factor *S* which "Glenshee" also carries, then no *Y* pollen can penetrate the styles of the mother (unless crossing over occurs in the male), and only non-yellow gametes will effect fertilisation. Five yellows to every ivory will thus appear in the F_1 where an 11 : 1 ratio was to be expected. Apparently no cross-overs have occurred in family 22/27, so that the particular factors for sterility and colour lie close together in the chromosome carrying them.

This interpretation of the unexpected ratio in family 22/27 awaits proof, but is advanced as the most plausible hypothesis in the light of present knowledge.

Family 37/27 also exhibits a marked deviation from expectation (*i.e.* 5 yellows : 1 ivory). In this instance the parents are not completely one-way incompatibles, although 27/24 is distinctly less effective as a male[1].

In the instances so far presented the results seem fairly amenable to interpretation. One cross remains, however, which gave colours intermediate between yellow and ivory.

TABLE V.

Fams. 2/28 and 3/28 (14/26 (yellow) *YYYyIi* × White Star (ivory) *yyyyIi*).

Colour	2/28	3/28	Total	Remarks
White	1	0	1	Faint streaks of ivory
Ivory	0	4	4	
Cream to ivory	2	1	3	
Cream	6	5	11	2/28—55 seeds, 50 germinated, 45 plants
Primrose to cream	8	6	14	3/28—47 seeds, 43 germinated, 39 plants
Primrose	19	13	32	
Deeper primrose	2	3	5	
Yellow of ♀	6	6	12	
Deeper yellow	1	1	2	
Totals	45	39	84	

Other pollinations in which the parents 14/26 and "White Star" were used gave yellows, ivories and whites, but when these two were crossed together a large array of intermediate, flavone colour forms appeared. From previous results it has been assumed that 14/26 is triplex for *Y* and heterozygous for *I*, and that "White Star" is nulliplex for *Y* and heterozygous for *I*. Hence no ivories or whites are to be expected in F_1.

[1] In this connection see also Crane and Lawrence (1929).

The individuals scored as "cream to ivory" and "primrose to cream" are (*a*) cream and (*b*) primrose at the base of the petals diffusing to ivory and cream at the tips respectively. Distribution factors are most probably responsible for these patterns. Reckoning the cream to ivories with the creams, and the primrose to creams with the primroses, we have 1? white, 4 ivories, 14 creams, 51 primroses and 14 yellows. If 14/26 is triplex for *Y* the four ivories (all in family 3/28) may have arisen from irregularity of division in the egg mother cells (as described on p. 138). The origin of the one white is problematical.

At first sight the presence of the cream and primrose individuals might be assumed to be due to the interaction of *Y* and *I*. Families 40/28 and 41/28 provide some evidence in this direction.

Family 40/28, derived from the crossing of *YYyyIi* and *yyyyii*, should consist of plants duplex and simplex for *Y* in the proportions 1 : 4. Half of these plants would be recessive for *I* and half heterozygous. As related on p. 137 an attempt was made to classify the 41 yellows in F_1, but the very slight differentiation encountered made this task impossible. Hence it follows that there is virtually no difference in flower colour when *I* is present (or absent) with one or two *Y* factors.

Family 41/28 confirms this suggestion. The 15 yellows should consist of *YI* and *Yi* plants in equal proportions. Differences were discernible in this family, but were too small to allow of any precise measurement. Again, when the yellows used as parents were examined it was found that the plants *Yyyyii* (Ideal), *YyyyIi* ($31^5/27$), *YYyyIi* ($22^9/27$) and *YYYyIi* (14/26) were phenotypically the same for colour.

All the yellows in families 40 and 41/28, and among the above parents, were deeper than primrose. If, therefore, the interaction of *Y* and *I* is responsible for the occurrence of cream and primrose, the factorial constitution of the parents must be such that the proportion of plants in F_1 scored as yellow should be in the minority, *i.e.* the recombinations *Yyyyii*, *YyyyIi*, *YYyyii*, *YYyyIi* must not exceed a sixth of the whole. The constitution of "White Star" is clearly *yyyyIi*. Whatever factorial constitution be assigned to 14/26, and scoring all genotypes known to be yellow, no combination will give less than half of F_1 as yellows.

Thus the interaction of *Y* and *I* is an insufficient explanation of the origin of the cream and primrose varieties. It is possible that a distribution factor, or factors, may in certain cases partially inhibit the production of yellow flavone, and in this connection the 14 creams : 51 primroses : 14 yellows may be a 1 : 4 : 1 ratio resulting from the tetra-

somic segregation of a factor partially suppressing the formation of yellow flavone.

The scoring of yellow, primrose and cream is also a difficult operation, since many minor differences occur, and the line of demarcation is sometimes rather arbitrary.

Summarising the breeding results for flavones—934 plants[1] were raised from 16 crosses involving the factor *Y* for yellow flavone. Table VI shows the agreement between observation and expectation in the segregation of

TABLE VI.

Parents	Progeny		Expectation		Goodness of fit
	Y	*y*	*Y*	*y*	P
Yyyy × *yyyy*	40	45	*42·5*	*42·5*	·50 to ·70
Yyyy × *Yyyy*	28	9	*27·7*	*9·2*	·90 to ·95
YYyy × *yyyy*	121	25	*121·6*	*24·3*	·90 to ·95
YYyy × *Yyyy*	200	22	*203·5*	*18·5*	·30 to ·50
YYYy × *yyyy* or *Yyyy*	437	6 (+ ? 1)	*444*	*0*	—

Six families show the inheritance of ivory flavone, and nine different parents were involved in making these crosses. 32/26 and 35/26 are recessive (*ii*) for ivory; "White Star," $22^9/27$ and $31^5/27$ are heterozygous (*Ii*); and 36/26, M 8 and M 2 are homozygous dominants (*II*). Three families were raised from crosses *Ii* × *ii* and a segregation of 30 ivories : 30 whites was obtained.

In the facts presented no characteristic tetrasomic segregation of *I* has occurred, and from the above results it seems probable that the segregation of ivory conforms strictly to expectation based on disomic inheritance of the factor *I*.

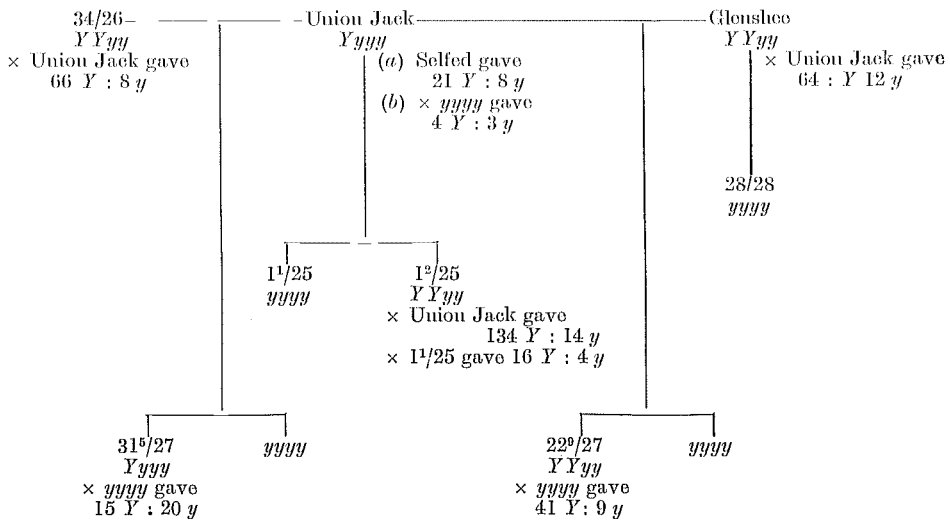
For reasons discussed later it is presumed that *I* is carried by four chromosomes whose relation is such that autosyndesis of I_1 with i_1 and i_2 with i_2 in individuals of the constitution *Iiii* gives typical disomic ratios.

As stated earlier, the main object of these experiments was the study of an abnormal condition of the flower, so that much of the data relating to flavone inheritance has been extracted from results obtained for another purpose. For this reason the number of F_2 and F_3 families raised has not been great, but in Table VII the relation of several plants is indicated in genealogical form.

It is noteworthy that the 5 : 1 and 11 : 1 ratios obtained in the segregation of *Y* are much nearer expectation based on a tetrasomic

[1] Omitting fam. 37/27.

TABLE VII.



inheritance of *Y* than any expectation upon disomic or octosomic inheritance. On p. 157 is appended for purposes of comparison a table showing the segregation expected from random assortment of 8 chromosomes carrying the factor A, in certain back-crosses in an octoploid.

(*b*) *Anthocyanins.*

Eleven families involving inheritance of anthocyanin have been raised, nine of which were from crossing. The parents used and the results obtained are shown in Table VIII. The numbers are small, but in certain instances seem to be significant.

TABLE VIII.

Fam.	♀	♂	Antho-cyanin	No antho-cyanin
—	2⁷124 (purple)	Selfed	20	0
—	Union Jack (scarlet)	Selfed	26	3
23/27 and 24/27 38/28 and 39/28	Union Jack (scarlet)	14/26 (yellow)	143	32
26/28 and 27/28	14/26 (yellow)	22⁸/27 (deep orange)	53	11
4/28 and 5/28	14/26 (yellow)	36/26 (magenta)	68	11
17/28	M 2 (dull scarlet)	White Star (ivory)	35	8
16/28	M 8 (purple)	White Star (,,)	23	4
8/28 and 11/28	36/26 (magenta)	White Star (,,)	33	6
26/27 and 31/27	34/26 (yellow)	Union Jack (scarlet)	60	14
32/27	Union Jack (scarlet)	Ideal (yellow)	6	2
30/27	Union Jack (scarlet)	32/26 (white)	7	0

In all the crosses where an F_1 of 20 or more plants was raised the ratio of coloured to non-coloured plants is approximately 5 : 1.

More critical evidence will be obtained by summing the crosses in which the same coloured parent has been used. Thus, summation of the individuals in the families where "Union Jack" was the parent gives 216 coloured to 48 non-coloured. Similarly in the families where 36/26 was used the total is 101 : 17. The expectations on a 5 : 1 basis are 220 : 44 and 98 : 20 respectively. It is singular that these results should agree so perfectly with the most probable expectation. Plants of the constitution *AAaa* for anthocyanin crossed to the quadruple recessive *aaaa* would give a 5 : 1 ratio.

Apparently "Union Jack" should be duplex for *A*, and if selfed should give 35 coloured to 1 non-coloured plant. The figures to hand, however, are contradictory since the actual result is 26 coloured to 3 non-coloured plants.

The evidence is thus too meagre to justify any conclusions on anthocyanin, and the instances are here recorded only because no detailed analysis of anthocyanin inheritance is at present contemplated in this series of experiments on *Dahlia*.

(3) CYTOLOGY.

Ishikawa (1911) reported the chromosome numbers of *D. variabilis* and *D. coronata* to be 64 and 32 respectively. Belling (1924) found *D. imperialis* to have 32 chromosomes.

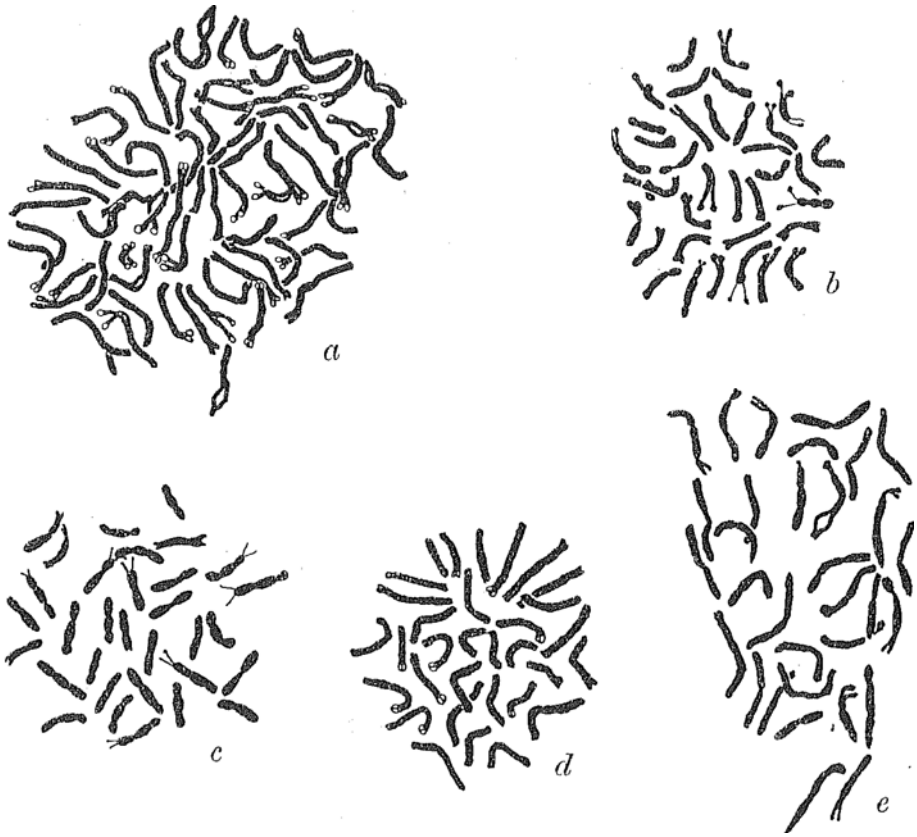
I have examined the following species and append their chromosome numbers.

D. variabilis	$2n = 64$	$n = 32$
D. Merckii	$2n = 36$	$n = 18$
D. Maxoni	$2n = 32$	
D. coccinea	$2n = 32$	$n = 16$
D. coronata	$2n = 32$	
Bidens atrosanguinea	$2n = 48$	$n = 24$
Hidalgoa Wercklei	$2n =$ ca. 31	

Maxoni and *imperialis* do not flower until November–December, and it has not been possible to get pollen mother-cell divisions of these two species. The other species all flower in the summer. Good pollen mother-cell counts of *coronata* have so far been unobtainable.

The somatic counts were made from root tips. *Variabilis* and *coronata* were fixed in Newton's modification of Flemming. The other

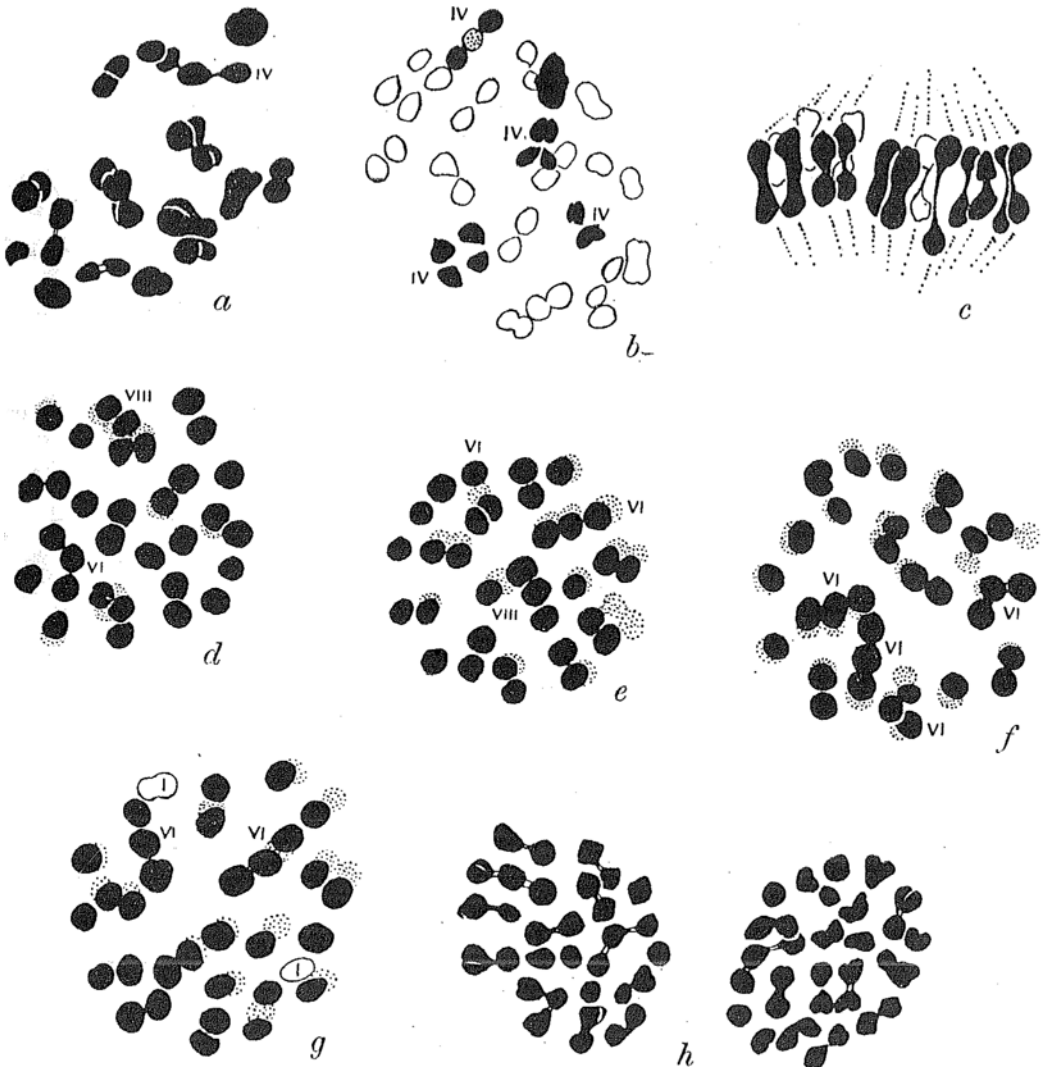
species were fixed in a solution of 60 c.c. 1 per cent. chromic acid, 20 c.c. 2 per cent. osmic acid and 25 c.c. 5 per cent. glacial acetic acid—which gave better results. Various fixatives were used for the pollen mother-cell material, the best results being obtained by Kihara's method (Carnoy 1 minute, followed by Flemming).



Text-fig. 1. Somatic divisions of polyploid *Dahlia* species. (*a*) *D. variabilis*, 64 chromosomes, octoploid; (*b*) *D. Merckii*, 36 chromosomes, hyper-tetraploid; (*c*) *D. Maxoni*, 32 chromosomes, tetraploid; (*d*) *D. coronata*, 32 chromosomes, tetraploid; (*e*) *D. coccinea*, 32 chromosomes, tetraploid.

By trimming off the outer bracts and quartering the capitulum the florets can be cut *en bloc* transversely or longitudinally. Quite good fixations were obtained by this method although the fixing of individual florets gives more consistent results. Smear preparations were tried without success.

Embedded material of root-tips was cut at 10 μ, and anthers at 14 μ. The sections were stained by Newton's gentian violet method. All



Text-fig. 2. Multivalent association of chromosomes in the octoploid *D. variabilis* ($2n=64$). *a–b*, diakinesis (*a*, incomplete); *c*, side view metaphase I; *d–g*, polar views, metaphase I; *h*, polar view of metaphase II.

drawings were made with the aid of an Abbé camera lucida, a Leitz 2 mm. objective (n.a. = 1·4) and a × 30 eyepiece. Magnification × 3700.

Every stage from early prophase to interkinesis has been seen in the anthers of one floret. In an individual locules the oldest pollen mother cells are at the base, the stages ranging from diakinesis to metaphase first division, metaphase to interkinesis, etc.

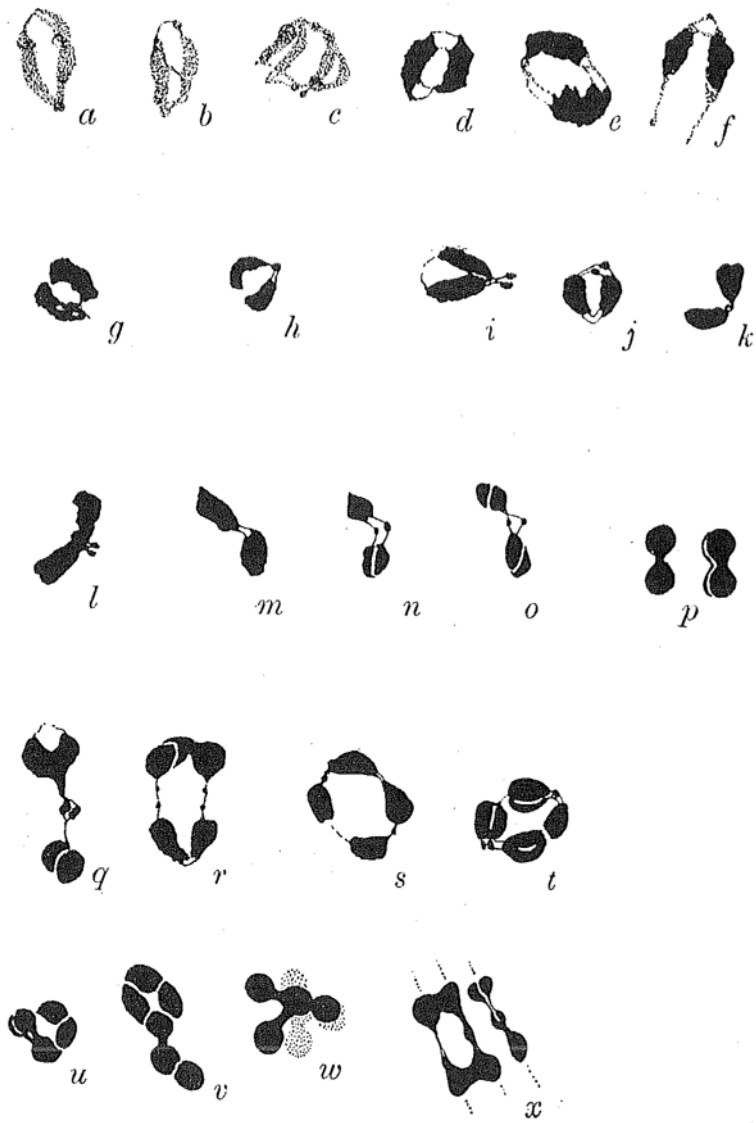
D. variabilis ($2n = 64$). Ishikawa (1911) reported *variabilis* to be a tetraploid species from the occurrence of "secondary" association of the chromosomes in the second divisions of the varieties examined. In the course of these experiments the chromosomes of eight varieties of the garden *Dahlia* have been counted in the pollen mother cells and found to be regularly 32 pairs. Every variety showed marked multiple association of the chromosomes at both first and second divisions. At metaphase the degree of this association varies from the closest intimacy (as between bivalent chromosomes) to the point where it ceases to be apparent. Quadrivalents, sexivalents, bivalents and octavalents occur in approximately this order of frequency in the first division, octavalents being by far the least frequent. Side views of metaphase plates exhibit regularity in the line up for division. At anaphase the movement of the chromosomes to the poles proceeds with much uniformity; laggards have never been seen. The strong association and large number of chromosomes make it difficult to determine and draw side views of divisions; nevertheless the numerous multivalents almost invariably divide regularly. A high percentage of the pollen is good.

The association of chromosomes eventually going to one pole is not necessarily the same as that of their respective partners, a differentiation possibly, but not necessarily, due to differences of genetical relationship.

In the second division the chromosomes are commonly associated in twos and threes.

It is highly probable that the so-called "secondary" association of bivalents at metaphase results from conjugation of homologous chromosomes at synapsis, during which I have seen chiasmata between what were evidently four chromosomes. Again, at mid to late diakinesis chiasmata have been clearly visible between bivalents and quadrivalents, though often they cannot be seen. The smallness of the chromosomes, coupled with the attenuated condition of the interconnecting strands, make it improbable that they will commonly be discerned in this material. In the other *Dahlia* species examined multivalents are decidedly less obvious at diakinesis than at metaphase.

It was this apparent discontinuity of the bivalents involved in multiple association at diakinesis that led Darlington (1928) in his studies



Text-fig. 3. Association of chromosomes in diakinesis of the octoploid *D. variabilis* $2n=64$. Illustrations selected from various meiotic figures. *a–b*, chiasmata between paired chromosomes; *c* and *e*, terminal chiasmata in quadrivalents; *d*, *f–o*, stages leading to the end-to-end association of bivalents at metaphase. A single terminal chiasma persisting; *p*, typical chromosome pairs at metaphase I, side view; *q–u*, quadrivalents; *v*, sexivalent; *w*, octavalent (polar view, metaphase); *x*, side view of quadrivalent and bivalent at early anaphase showing sub-terminal and sub-median chiasmata respectively.

on *Prunus*, to ascribe the phenomenon to a post-synaptic influence. In more recent work on *Hyacinthus* and *Tulipa* Newton and Darlington (1929) have shown in detail the nature of conjugation between more than two homologous chromosomes. The association, at the reduction divisions, of more than two homologous chromosomes, is also discussed by Meurman (1929) who concludes from his study of *Prunus laurocerasus* that this pairing is the result of a real synaptic conjugation of the chromosomes. It is probable that the tenuous nature of the connecting strands between small chromosomes prevents discernment of the physical continuity existing among the individual components of multivalent associations. In more suitable material this continuity can be demonstrated.

The chromosomes of *Dahlia* are too small to permit of a critical study of synapsis. Conjugation is evidently parasynaptic, the single threads lying side by side in various degrees of association.

The subsequent development of bivalents and multivalents is figured in Text-fig. 3. At pachytene the paired chromosomes lie side by side, but as the chromatin contracts the chiasmata are either broken or pushed along the chromosomes until terminal chiasmata alone remain. The paired chromosomes now begin to swing apart at one end, and finally lie end to end with a single chiasma between them. The terminal chiasmata are visible at every stage of diakinesis, and, though lost sight of at metaphase, sometimes become evident again as the paired chromosomes pull apart in early anaphase.

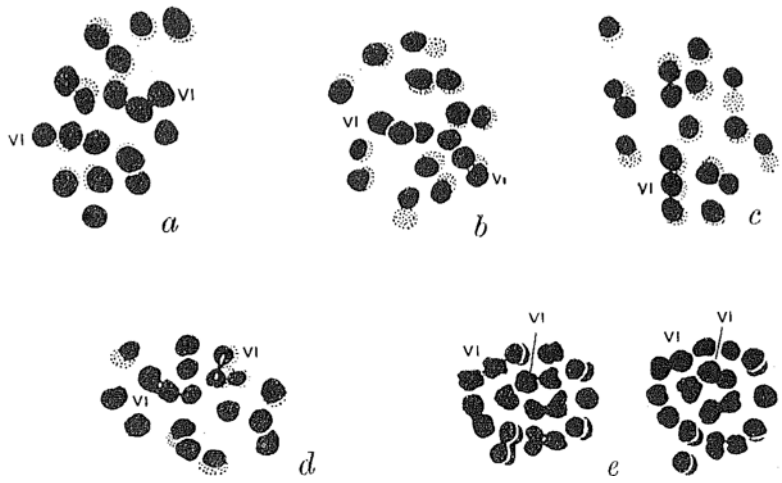
The development of the multivalent bodies is naturally more variable. From earliest diakinesis multivalent associations may be discerned. The pairing between the components, each to each, is variable, and probably results from differences of chiasma formation and development.

Counts made in diakinesis reveal many less than 32 bodies; as few as 13 and 14 have been counted. The average number would seem to be somewhere between 15 and 20. These bodies differ in size, and as diakinesis proceeds the larger are seen to be compound, their components separating to give rise to the characteristic multivalent bodies seen at late diakinesis, and to the bivalents associated at metaphase.

D. Merckii ($2n = 36$) is a very distinct species and the only one I have seen with fertile ray florets.

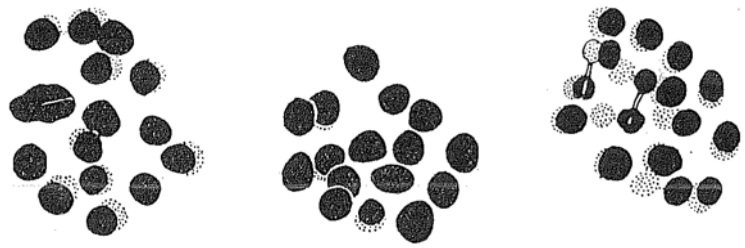
The pollen mother cells are noticeably smaller than in *D. variabilis*. Quadrivalents are seen in diakinesis, and especially at metaphase of the first division. Each of the two extra pairs of chromosomes is homologous with a quadrivalent group, forming two sexivalent associations. These

may be seen in most metaphase plates. It is noteworthy that the self-fertility of *Merckii* is not appreciably affected by the addition of these four chromosomes.



Text-fig. 4. *D. Merckii.* A hyper-tetraploid species ($2n=36$). *a–d*, polar views of metaphase I showing sexivalent, quadrivalent and bivalent association of the chromosomes; *e*, polar view of late anaphase I.

D. coccinea ($2n = 32$). Multiple association is less noticeable than in any of the species examined, but many quadrivalents have been observed. The pollen mother cells are about the same size as those of *D. Merckii*.



Text-fig. 5. Bivalent and quadrivalent association of chromosomes in the tetraploid *D. coccinea* ($2n=32$). Polar views of metaphase I.

Bidens atrosanguinea ($2n = 48$). This plant was examined because of the superficial resemblance it bears to *D. Merckii*. A root-tip count gave the somatic number as approximately 48, which was later confirmed by a pollen mother cell count of 24. Considerable irregularity of the

divisions was apparent and multiple association was also marked. I have attempted to cross *Bidens atrosanguinea* and *Dahlia Merckii* but without success. The *Gardener's Chronicle* (1910) records that a Mr Gunbleton, of Cork Co., raised 500 seedlings of *B. atrosanguinea*, 25 of which were white. The others were purple, rose and pink.

Hidalgoa Wercklii ($2n$ = ca. 31). This plant was examined because of its systematic relation to *Dahlia*. The peculiar sexual differentiation of the capitula seemed to indicate a polyploid and hybrid constitution, and the cytological evidence bears this out.

DISCUSSION.

(1) General observations.

The identification of characters which would definitely separate *D. variabilis* from all other *Dahlia* species in any systematic scheme has always been a difficulty.

Hemsley (1879), in addition to discussing the vague characteristics of the garden *Dahlia*, gives an excellent account of the variation to be found in the genus, and mentions a few of the many conjectures which have been advanced as to the origin of *D. variabilis*. Undoubtedly, much of the early confusion arose from careless naming of plants by horticulturists, since the original species introduced were perfectly distinct. Thus, *coccinea*, grown side by side with *variabilis*, can in no wise be confused with it, although these two have been subjects of frequent contention.

Cytological examination has made the problem of relationship and phylogeny easier to solve, though much more work is necessary to warrant any certainty of opinion.

The occurrence of the two colour series in *variabilis*, but not in any other species, is especially significant in the light of the cytological evidence. Morphologically these two colour groups exhibit many characters in common, though certain features are peculiar to one group or the other. Thus the giant tree Dahlias are all in Group I (ivory, magenta or purple flowers) and are late-flowering species; the variation in flower colour in Group II is mainly, if not completely, quantitative—a variation of intensity, whereas, in Group I, there are two distinct anthocyanin colours, the first a rosy pink, the second a violet, both occurring in varying intensities.

All the species grown in the course of these experiments have glaucous stems, are tetraploids, and are self-fertile. The self-fertility has not been

critically tested, but there are indications that it may be somewhat variable.

The distribution of *Dahlia* species is confined to the central American region of Colombia, Mexico and Guatemala, the majority of plants collected coming from Mexico. Hence the relative proximity of related species would increase the chances of interspecific hybridisation, the probable result of which would be the evolution of forms showing considerable similarity.

The origin of polyploid plants by hybridisation and under experimental conditions has only been recorded a few times. *Primula kewensis*, Newton and Pellew (1926), is known to be a sterile diploid hybrid from *P. floribunda* × *P. verticillata*, and it is also known that somatic doubling of the chromosome number restored fertility and gave rise to a fertile race of *P. kewensis*. In *Nicotiana glutinosa* × *N. Tabacum*, Clausen and Goodspeed (1925) obtained "reasonably fertile" polyploids as the result of doubling. Karpechenko (1927) found that though the diploid *Raphanus-Brassica* hybrids were sterile, they gave rise to tetraploids which were highly fertile. Darlington (1928) has drawn attention to these and to other examples, and points out that there appears to be an inverse correlation between the sterility of a diploid and the fertility of a tetraploid to which it gives rise.

More recently, Buxton and Newton (1928), and Jørgensen (1928) have also published results demonstrating this inverse correlation.

In the former case hybrids between *Digitalis purpurea* and *D. ambigua* were practically sterile, but from the selfing of F_1 individuals fertile plants with double the number of chromosomes were obtained, and these were shown to have arisen from the fusion of unreduced gametes.

Jørgensen, working with *Solanum*, artificially induced the doubling of somatic cells of fertile diploid species. The polyploid plants subsequently raised showed reduced fertility. He also raised a sterile hybrid, *S. nigrum* × *luteum*, which, upon doubling of the somatic chromosome complement, gave fertile tetraploid shoots and offspring.

The accumulating evidence thus appears to establish the validity of the above generalisation. The occurrence in *D. variabilis* of two distinct colour series, only one of which is found in any *Dahlia* species with half the chromosome number, suggests that hybridisation has taken place between two tetraploid species—one belonging to Group I, the other to Group II. From Darlington's rule it may be presumed that the general fertility of *D. variabilis* indicates a relatively infertile tetraploid parent,

fertility being restored upon the occurrence of doubling of the hybrid chromosome complement.

Thus pairs of bivalent chromosomes, which, though present in one of the immediate tetraploid species, were not represented in the other, would, after doubling of the chromosome complement of the sterile hybrid, give a tetrasomic segregation if random assortment prevailed. The inheritance of yellow flavone is in accordance with this scheme.

On the same basis, disomic inheritance of ivory, if substantiated, indicates that only autosyndesis occurs among the chromosomes concerned, *i.e.* I_1 pairs with i_1 and i_2 with i_2

Therefore, if we tentatively consider the sterile, tetraploid precursor of *variabilis* as having the chromosomes I_1i_2, which do not ordinarily pair—then doubling of the chromosome complement $\left(=\frac{I_1i_2}{I_1i_2}\right)$ must have been followed by (1) allosyndesis and crossing-over, or (2) loss mutation giving rise in either case to i_1i_2 gametes.

From the considerable number of quadrivalents seen at metaphase it may be predicted that other characters will show a tetrasomic segregation.

(2) Relation of flavones to anthocyanins.

Onslow (1925), discussing the colour series in various plants, remarked on the similarity of those of *Antirrhinum majus* and *Dahlia variabilis*. In *Antirrhinum* the factor *Y* simultaneously produces yellow flavone in the lips and ivory in the tube. *I* is "dominant" (*i.e.* epistatic) to it and suppresses the formation of yellow flavone in the lips—ivory taking its place. Absence of *Y*, though *I* or any other colour be present, gives white. Red anthocyanin on yellow gives bronze; on ivory, rose. Magenta anthocyanin on yellow gives crimson; on ivory, magenta. There are no intermediate colours as in *Dahlia*, although tinged varieties occur. We see then that (1) one factor controls the production of the two flavones; (2) a single anthocyanin factor produces pigment in the presence of either flavone; (3) no anthocyanin is formed if flavone is absent. With regard to this latter observation Onslow remarks that "the development of anthocyanin and flavone (in *Antirrhinum*) may depend on some common basal metabolism for production of aromatic substances, *i.e.* if flavone is not formed, neither is anthocyanin." This view is supported by the experiments on *Dahlia*.

So far it is clear that there are two independent flavone colours, absence of which gives white (no flavone). Anthocyanin is formed only when one (or both) of the flavones is present.

What is the relation of anthocyanin to these flavones? Either (1) there is one anthocyanin pigment which, though formed only in the presence of flavones, is otherwise independent, and in combination with yellow and ivory gives scarlet and purple flower colours respectively; or (2) a distinct anthocyanin is formed in the presence of each flavone.

Evidence as to the behaviour and inheritance of anthocyanin in *Dahlia* is still fragmentary, but there are indications that it is tetrasomic and that, possibly, the factor operates in the presence of either flavone as in *Antirrhinum*. If this is the case the quality of the flower colour will be dependent upon the flavone colouring, and will exhibit a variation parallel with that of the flavone, whatever may be the relation of anthocyanin to flavone.

Willstätter (1915), cited by Onslow, has found that certain deep brown-red varieties of *Dahlia* form cyanin, but pelargonin is formed in the scarlet-red varieties. Both pigments were found in a dark violet variety. As stated earlier in this paper the "scarlet" pigment in these experiments always gives a brown-red reaction with ammonia, the purple pigment alone giving the characteristic blue-green reaction. The evidence therefore points to the presence of more than one anthocyanin pigment in *D. variabilis*, and should there be found only one factor for anthocyanin production then it becomes highly probable that a definite relation exists between the production of flavones and anthocyanins.

Wheldale (1913) has reported the yellow flavone, in *Antirrhinum*, to be luteolin and the ivory to be apigenin, while Schmid and Waschkau (1928) isolated and analysed the yellow pigment of *Dahlia* in quantity and stated it to be identical with apigenin. Certain yellow Dahlias, however, have been shown to carry ivory which is obscured by yellow (at least in some instances), and the question therefore arises as to which of the two pigments was isolated and identified, especially as apigenin is said to be the ivory pigment in *Antirrhinum*.

It may be surmised that in *Dahlia* the two flavones are luteolin and apigenin and that the two anthocyanins are pelargonin and cyanin, but pending further investigation the identity of these pigments remains uncertain.

(3) HISTORICAL AGREEMENT.

It is noteworthy that records of the early varieties obtained by plant breeders when *Dahlia* was first introduced into Europe substantiate the colour scheme outlined in this paper. The earliest figures of *D. variabilis* were published by Francisco Hernandez in 1615 and 1649. There seems

little doubt that long before its introduction into Europe it had been cultivated in Mexico, where it was a well-established species showing considerable variation. "Tubers" of two varieties of *D. variabilis*—a purple, *pinnata*, and a crimson, *rosea*, were first sent from Mexico to Madrid in 1789.

The purple flowered in October 1789, the crimson a year or two later. Cavanilles figured and described them in 1791 and 1794 respectively. In 1802 *plants* of each were transferred from Madrid to the Jardin des Plantes at Paris. In 1804 *seeds* from the three original plants were sent to Lady Holland, who flowered them the following year. In 1803 Mr Woodford also flowered a *plant* of Cavanilles' *rosea* obtained from Paris. Johnson and Turner (1847) say that the plants at Madrid were in the Royal Garden for a long time without any indication of change, and that after they were spread through Europe some years elasped before any extensive increase of variation took place. At Holland House seeds were saved and sown from the plants received in 1804 and all of them gave rise to plants of some shade of purple or crimson. Controlled cross-pollination was apparently not practised until after 1805–6.

In 1818 Sabine remarked that he had never seen a pure scarlet and that all coloured varieties seemed to have a tinge of purple. In 1826 Smith, referring to Sabine's observation, says: "the new scarlets are however perfectly pure."

Thus quite a number of years passed before pure scarlets were obtained by selection of seedlings from the original stock. Cavanilles' purple and crimson probably had ivory and cream grounds respectively. If the latter were triplex or quadriplex for a partial inhibitor of *Y*, it would, in view of the small number of plants available for crossing in the early years, have taken a considerable time to procure a variety with a pure yellow ground such as the pure scarlets have. Moreover there would have been a tendency to increased delay owing to the self-sterility of *D. variabilis*—a characteristic favouring heterozygosity.

SUMMARY.

(1) Flower colour in *Dahlia* is the expression of two series of soluble pigments: (*a*) flavones, (*b*) anthocyanins.

(2) With the exception of *D. variabilis*, *Dahlia* species may be arranged in two flower colour groups: (*a*) ivory, magenta or purple; (*b*) yellow, orange or scarlet. *D. variabilis* unites both series within itself.

(3) In *D. variabilis* ivory and yellow are flavone colours governed by the independently inherited factors *I* and *Y*. The inheritance of *Y*

is tetrasomic, arising from random assortment of four homologous chromosomes. The inheritance of I, so far, has been disomic only. Autosyndesis of four chromosomes, I_1 with i_1 and i_2 with i_2, would give this result, but further work is necessary to establish this assumption.

(4) Orange and scarlet flowers have yellow flavone grounds; magenta and purple have ivory grounds; crimson flowers have intermediate coloured flavone grounds. Anthocyanin is formed only in the presence of flavones.

(5) Somatic mutations involving the loss of flavone in the presence of anthocyanin are shown to bring about corresponding changes in flower colour. The relation of flavones and anthocyanins is discussed.

(6) *D. coccinea*, *D. coronata*, *D. imperialis* and *D. Maxoni* are tetraploids with 32 chromosomes; a fifth, *D. Merckii*, has 36, and each of the two extra pairs is homologous with a quadrivalent group, forming two sexivalents at metaphase of the first division. *D. variabilis* is an octoploid species with 64 chromosomes. Multiple association of the chromosomes at meiosis is seen in these species, and is very pronounced in *variabilis*.

(7) Bivalents, quadrivalents, sexivalents and octavalents are to be seen at metaphase of the first division in *D. variabilis*. These multivalent associations seem to result from the conjugation of homologous chromosomes at synapsis, and evidence is advanced in support of this view.

(8) From the morphological, genetical and cytological observations it is suggested that the octoploid *D. variabilis* is the derivative by doubling of the chromosome complement of a relatively infertile hybrid of two tetraploid species, one belonging to the ivory-magenta-purple and the other to the yellow-orange-scarlet colour groups.

TABLE IX.

Octosomic segregation of a factor A and recombinations in back-cross to a_8.

Random assortment of chromosomes.

	Gametes					Zygotes					
Parent	A_4	A_3a	A_2a_2	Aa_3	a_4	A_4a_4	A_3a_5	A_2a_6	Aa_7	a_8	Ratio of A to a
A_8	∞	—	—	—	—	∞	—	—	—	—	—
A_7a	35	35	—	—	—	1	1	—	—	—	—
A_6a_2	15	40	15	—	—	3	8	3	—	—	—
A_5a_3	5	30	30	5	—	1	6	6	1	—	—
A_4a_4	1	16	36	16	1	1	16	36	16	1	69 : 1
A_3a_5	—	5	30	30	5	—	1	6	6	1	13 : 1
A_2a_6	—	—	15	40	15	—	—	3	8	3	11 : 3
Aa_7	—	—	—	35	35	—	—	—	1	1	1 : 1
a_8	—	—	—	—	∞	—	—	—	—	∞	—

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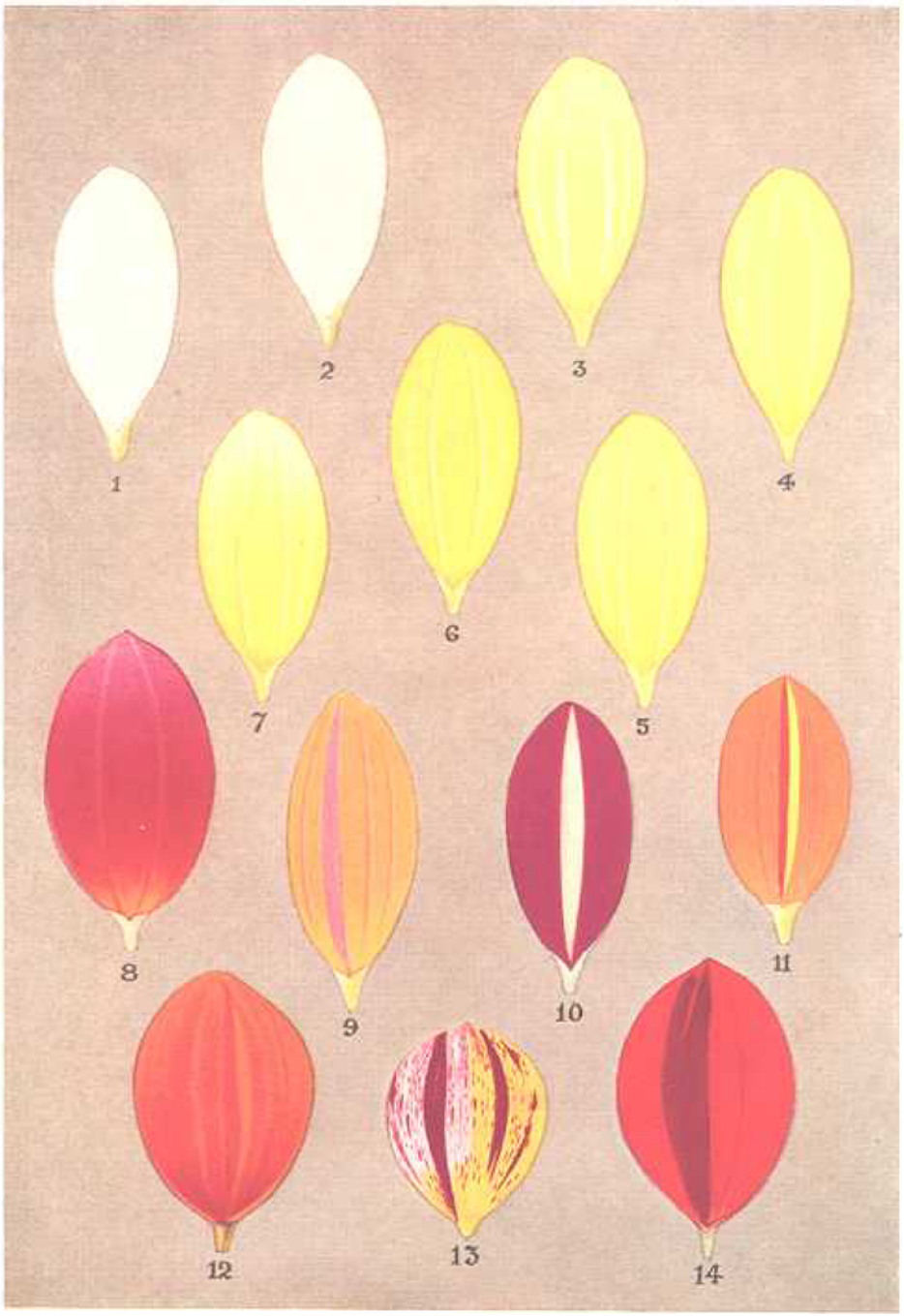
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EXPLANATION OF PLATE XII.

Flower colour and somatic variation of same in *Dahlia variabilis*.

Fig. 1. White (no flavone).

Figs. 2–6. The flavone ground colours, viz. ivory, cream, primrose, yellow and deep yellow respectively. (Figs. 2 and 3 somewhat accentuate the difference between these two colour types.)

Fig. 7. Yellow shading to cream.

Fig. 8. Anthocyanin on ground as in Fig. 7.

Fig. 9. Loss of yellow flavone from sector of buff petal, giving magenta stripe.

Fig. 10. Loss of anthocyanin from sector of purplish petal, revealing ivory ground.

Fig. 11. Loss of anthocyanin from sector of orange petal, revealing yellow ground. The adjacent sector is doubly coloured.

Fig. 12. Sectors showing dilution and intensification of anthocyanin.

Fig. 13. Mosaic distribution of anthocyanin on yellow (normal) and ivory (loss of yellow) grounds. The anthocyanin appears crimson on yellow and purplish on ivory. From the var. "Dorothy."

Fig. 14. Loss of yellow flavone from sector of crimson-scarlet petal, giving purple stripe. From the var. "Union Jack."