ANALYSIS OF FLOWER COLOUR IN *PHARBITIS NIL.*

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(With Plate V and Six Text-figures.)

CONTENTS.

INTRODUCTION.

THE researches in relation to the genetics of *Pharbitis Nil* have been carried on for some twenty years. Pioneers in this field are Toyama and Takezaki. Prior to them, Yasuda made crossing experiments with this plant without knowing of Mendel's paper. In the course of these researches, especially during the last fifteen years, there have been numerous publications on this subject.

The Japanese morning glory exhibits abundant variation, especially in the flower colour itself and the corolla patterns, furnishing, together with the other characters, favourable material for our investigation. Owing to the complexity of the variation exhibited, and also to the delicacy of the flower colour when exposed to sunshine, its study is rather troublesome. Contributions to the genetics of flower colour are due especially to Hagiwara, Imai, Miyake, Miyazawa, Takezaki and Tanaka. In this paper are described the behaviour of certain genes affecting the flower colour, leaving some others for future investigation. Experimental data on the general flower colours will be omitted here.

The writer wishes to make acknowledgment to Prof. K. Miyake for his useful help and valuable advice, to Messrs B. Kanna and K. Tabuchi for their friendly assistance, and also to Prof. R. C. Punnett for his important suggestions in the preparation of this manuscript.

EXPERIMENTAL ANALYSIS WITH FLOWER COLOUR.

WHITE FLOWERS.

The genetics of the white flowers of *Pharbitis Nil* is complicated, owing to the occurrence of several forms differing in their genotypes. The common genes found in white flowers are white-1 and white-2, the dominant allelomorphs of which are complementary for the production of colour. The genetic relation of these two genes has been worked out and verified by Takezaki (1916), Imai (1921) and Hagiwara (1929 *c*). Another gene, white-3, which results in a white flower with white seed, was studied by Miyazawa (1923) and Imai (1927 *a*). The dominant allelomorph of white-3 is also complementary for the production of colour with the other two dominant allelomorphs of white-1 and white-2. Imai and Tabuchi (1929) obtained a white mutant in a duskish family, the white flower being transmitted as a dominant character to duskish. Later experiments show that this white is due to the combined effect of the genes rayed and duskish; that is, rayed results in a white flower working on the duskish component by eliminating the colour from the corolla. Hagiwara (1929 *b*) records the occurrence of dominant white flowers in his study. According to him, his dominant whites are produced by duplicate genes for the complete inhibition of the flower colour. A close examination of his data, however, reveals the fact that they seem to have contained some contaminated individuals which caused him to draw the conclusion for the so-called dominant whites. Hence we have as yet no completely dominant white flowers in this plant. Rayed produces a dominant white flower, but only when it works on duskish. The effect of the gene itself, however, is to reduce the colour of the corolla.

White-1. White-1 (**w1**) is on a coloured stem. The flowers and their tubes are white, notwithstanding the fact that the stems are coloured.

The stem colour of white-1 varies according to the genic composition for the flower colour, though this of course is not actually developed. Owing to their coloured hypocotyls and stems, white-1 seedlings cannot be distinguished before flowering. The writer made several intercrosses between different strains of white-1, but obtained only white-1 in F_1 and later generations. By crossing experiments white-1 is proved to be a simple recessive to normal.

White-2. The most common type of white flowers is white-2 (**w2**), which has green stems. Sometimes, however, the corolla is tinged with a very faint colour. The flower tubes are either white or coloured. White-2 may be identified in seedlings, according to their hypocotyls. White-2 is recessive to normal, and its dominant allelomorph is complementary in the production of flower colour with **w1**. The double recessive, **w1 w2**, has always a white tube and cannot be distinguished phenotypically from a white-tubed **w2**. When white-2 of coloured tube is crossed with white-1, the **w2** and **w1 w2** segregates can be distinguished by phenotypes according to their tube colours. The genotype of the so-called white-2 is not always simple, because intercrosses between white-2 give at times coloured F_1, with a 9 : 7 segregation in F_2. Its genetics require further investigation.

White-3. White-1 and white-2 bear black seeds, while white-3 (**w3**) produces white seeds. White-3 has green stems and white tubes, and behaves as recessive to normal. The dominant allelomorph of white-3 is complementary with either **w1** and **w2** in the production of flower colour. White-1 and white-2 are hypostatic to white-3.

FLOWER HUES.

According to old literature, the prototype in the flower colour of our morning glory is "dilute blue," from which many divergent forms appeared in the course of evolution under cultivation. In this paper, the flower shown in Plate V, fig. 1, is regarded as the standard or normal. In the comparison of the flower hues, it seems better to take their intense colours rather than their dilute ones, the expression of the respective colours being strong in the former. The intense blue flower is "Blackish Violet"[1] (Plate V, fig. 2), according to Ridgway's *Color Standard and Color Nomenclature* (1912). The other various hues founded in the varia-

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[1] The precise identification of the colour in general is rather difficult, owing to minor variability exhibited in a given flower colour. Therefore the identification shows the nearest colours of the respective standards. When the tube is white (tube-white) the flower colour is less reddish, or Ridgway's "Deep Blue-Violet" in this case.

tion of the flower colour are effected by several mutant genes. A number of papers have been published on flower colour, of which we may cite especially those of Miyazawa (1918, 1921), Imai (1919) and Hagiwara (1923).

Purple. The recessive gene purple (**pr**) makes blue into purplish, or Ridgway's "Cotinga Purple" in its intense variation (Plate V, fig. 3). The dominancy of blue over purple is apparently complete.

Magenta. This recessive variant blooms into magenta in colour or Ridgway's "Rood's Violet" in its intense variation (Plate V, fig. 4). Normal has brownish hairs on stems and leaves, whereas magenta (**mg**) manifests white hairs. In the white flowers, even in those having green stems, the identification of the gene magenta can be made by this trait. The hypocotyls and stems of normal are dark purplish in colour, while they are dark red on magenta plants. The double recessive, **pr mg**, is "red," or Ridgway's "Amaranth Purple" in its intense variation (Plate V, fig. 5).

Dusky. Dusky (**dy**) is a recessive gene, producing dull colour, or Ridgway's "Dark Hyssop Violet" in its intense variation (Plate V, fig. 6). In combination with **pr** and **mg**, it results in the corresponding colours; namely, Ridgway's "Deep Livid Purple" by **pr dy**, "Corinthian Purple" by **mg dy** and "Neutral Red" by **pr mg dy**.

Duskish. This variation was first described by Hagiwara (1928, 1929 *a*). The expression of duskish (**dk**) is more dilute than dusky, and it exhibits a distinct hue, viz. Ridgway's "Vinaceous Purple." The gene duskish is mutable in its property. By the combination with **pr** and **mg**, duskish also results in the corresponding variations in the flower colour. Plate V, fig. 7, is **pr mg dk**. Duskish is epistatic to dusky.

FLOWER TONES.

The variable intensity of the flower colour in combination with its variable hues leads to much complication. According to old literature, the original type in regard to the flower tones is "dilute." This shows roughly the grade of intensity, but we have several "dilute" flowers differing genetically. The writer took the most common "dilute" or Ridgway's "Bradley's Blue" (Plate V, fig. 1) as the standard type in his study, this grade being presumably regarded as the prototypic blue form. In this connection we may cite especially those papers worked out by Miyake and Imai (1920) and Miyazawa (1921).

Intense. Intense (**i**) (Plate V, fig. 2) darkens the flower colour, and behaves as a recessive to normal.

Light-1. Two light flowers due to different genes have been detected. Light-1 (**lt1**) (Plate V, fig. 8), which is recessive to intense, is linked rather closely with yellow and dusky (Imai, 1931).

Light-2. Light-2 (**lt2**) is the other lightening gene, working as recessive to intense. The gene is not located on the yellow chromosome.

Dilute. Dilute (**D**) is dominant to normal, reducing the flower tone to Ridgway's "Amparo Blue" (Plate V, fig. 9).

Tinged. Tinged (**tg**) is a recessive character to normal, reducing the flower tone to a considerable degree (Plate V, fig. 10).

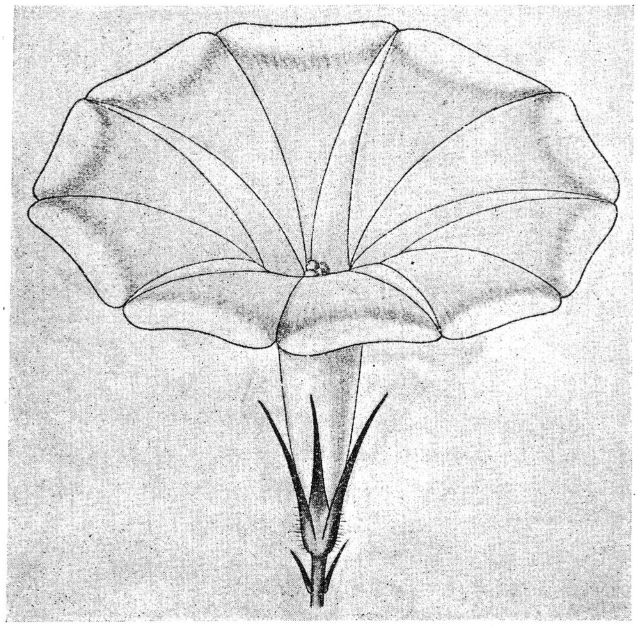
FLOWER PATTERNS.

Besides self-coloured flowers containing various hues and tones as described above, we have a number of different flowers marked with particular patterns. The distribution of the colour is restricted in some regions of the corolla, the restriction, however, occurring either on its definite or indefinite parts. In the latter case, a marked variation occurs in the distribution of variegation. Several genes affecting the white margin of the corolla are known, but are not described here. Hagiwara (1926) and others have analysed the genetic composition of several flower patterns.

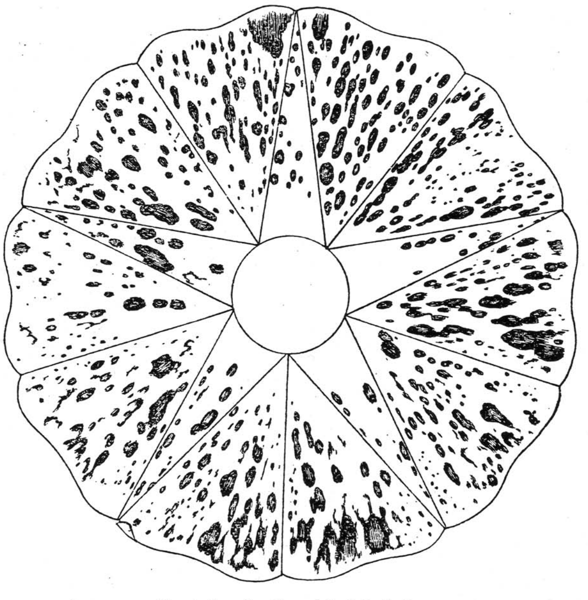
Speckled. Speckled (**sp**) is a recessive flower, having fine spots distributed over the corolla (Plate V, fig. 11). The character shows much variation, both qualitative and quantitative. It was first studied by Tanaka (1915), and later by Imai (1921). It restricts the extension of the anthocyanin colour in the corolla, leaving a yellowish or white background. If the speckled flowers have coloured tubes, the restriction of the pigment occurs also on them. Thus coloured-tubed white-2 flowers carrying speckled have white corollas with speckled tubes. Various colours occur with speckled restriction.

Speckled-reduced. Speckled-reduced (**sp–r**) works as a recessive modifier for speckled, and it results in almost non-spotted flowers (Imai, 1921). The speckled-reduced flowers have sometimes a few fine spots on the otherwise yellowish, pale (Plate V, fig. 12) or white background. The yellowish background colour is due to the flavone pigment, and its intensity is affected by the gene intense.

Faded. Faded (**fd**) is a recessive variation, the flower colour being faintly faded (Imai, 1921). The hypocotyls of the faded seedlings are green, except on their lowest parts, which are coloured very dilutely. When faded flower is marked with a white margin, the inner region of which is rich-coloured, there is formed a ring on the corolla (Text-fig. 1).



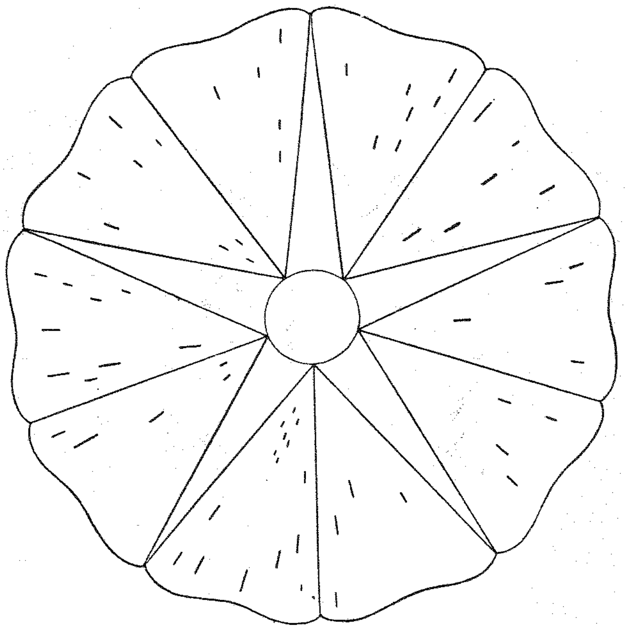
Text-fig. 1. Faded (margined).



Text-fig. 2. Speckled faded.

The speckled faded flower has faded speckles with whitish centres (Text-fig. 2).

Smeary. Smeary is an intermediate form approaching faded, the flower being shaded dilutely into Ridgway's "Light Violet-Blue" in its intense blue variation. When smeary flower has a white margin, a heavy tone appears on the inner adjoining region to the white margin (Plate V, fig. 15). The stems of smeary are fully coloured. Smeary (**fd^s**) is recessive to normal, but dominant to faded, the three forming an allelomorphic series.



Text-fig. 3. Flecked.

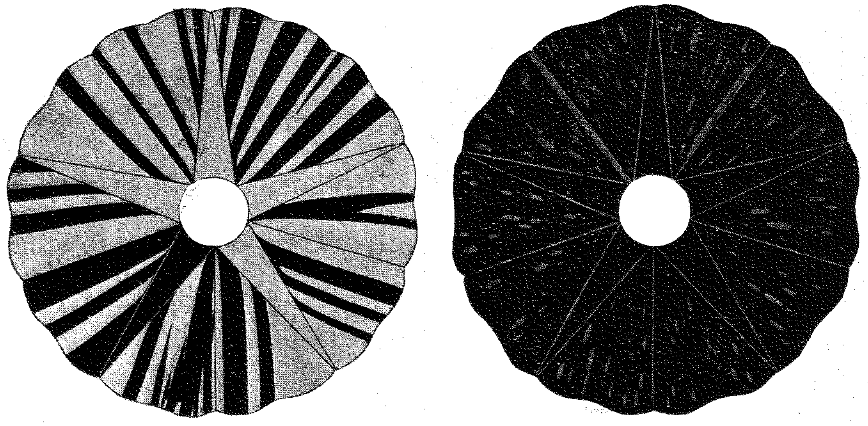
Rayed. Rayed is applied to a flower having coloured rays, the colour fading away in the regions between the rays (Plate V, fig. 13). Rayed (**Ry**) is an old dominant variation, and also reappeared in the writer's material (see later). The old and new Rayed gave the same results in crossing experiments. Plate V, fig. 14, is **sp Ry**.

Flecked. Flecked flower is marked with anthocyanin flecks on the white background (Text-fig. 3). The flecks vary to a considerable extent in their size as well as in their distribution. The gene flecked (**fl**) is mutable, changing at times to its dominant fully coloured condition.

Lined. As stated elsewhere (Imai, 1929), we have a recessive variegated flower form called striped. The similar character named Lined (**Ln**), however, is dominant to normal. In Lined, deeply coloured variegation occurs on the otherwise dilutely coloured background of the corolla (Plate V, fig. 18). The amount of variegation varies to a great extent.

Striated. Striated (**sa**) has dilute flower colour, with fine stripes, the colour fading off towards the margin of the corolla (Plate V, fig. 17). Sometimes variegation occurs more plainly, distributed over the corolla. Striated gives a rather soft appearance among the variegated flowers by its delicate stripes outlined on the faintly coloured background. The gene striated is recessive to normal.

Blizzard. Blizzard flowers have deeply coloured stripes on a lightly coloured background, and frequently show sparsely scattered whitish spots (Plate V, fig. 16). Hagiwara (1926) found Blizzard to be dominant to normal, and also discovered that two complemental genes Blizzard-1 (**Bz1**) and Blizzard-2 (**Bz2**) were concerned in the production of the character. Through the interactions of these two genes the amount of variegation fluctuates to a very considerable extent (cf. Text-fig. 4), leading at times to the production of flowers which are almost without variegation.



Text-fig. 4. Two forms of Blizzard, showing fluctuation.

MUTATING CHARACTERS.

Several mutating genes have been detected in the Japanese morning glory. The writer (Imai, 1927 *b*) has already studied the mutability of cream, and will now give an account of the variability of the genes flecked and duskish.

FLECKED PEDIGREE.

Source of material and inbreeding experiments.

In 1924 the writer was given a plant in a pot by his late aunt, Mrs T. Shimizu. This plant, the origin of the present study, was flecked, being characterised by white flowers with fine reddish flecks (Text-fig. 3). It showed itself to be a mosaic, bearing branches with self-coloured flowers of "red" (genetically, purple magenta) colour. The stems to which flecked flowers are attached are green, whereas those to which self-coloured flowers are attached are coloured dark red. In 1925 the writer bred the progenies from both parts, flecked and normal, on the original mosaic plant, which, however, produced rather few seeds, because of the fact that the plant was cultivated in a small pot. The offspring contained one normal and seven flecked from flecked flowers, and fourteen normal and six flecked from normal flowers on the original plant. These data, though small in number, show, first, that the gene flecked is mutable, and, second, that the normal tissues which changed from flecked are produced by vegetative mutation. These facts have been verified by the experiments made in subsequent years, and, further, showed more complication in the behaviour of flecked. Two series of pedigree culture experiments were made in 1926; one relates to the offspring of family *S*, which was derived from normal, self-coloured flowers of the original plant; and the other to family *F*, from flecked flowers. The offspring of these families are given in Table I.

TABLE I.

Data collected in the pedigree culture of flecked in 1926.

Mother plant		No. of families	+	fl	Total
Family *S*	Normal	2	55	—	55
		5	152	44	196
	Flecked	2	2	88	90
Family *F*	Normal	1	22	6	28
	Flecked	5	4	90	94

The offspring of family *S* shows that normal plants sprung from the normal part on the original mosaic plant consist of some individuals homozygous for normal and others heterozygous for flecked. The offspring of family *F*, together with those of family *S*, confirm the earlier results. The fourth generation was raised from family *F* in 1927, and Table II contains the data gathered in that year.

The amount of variegation varies widely, producing a few white

TABLE II.

Data collected in the pedigree culture of flecked in 1927.

Mother plant	Family no.	+	fl	"White"	Total
Normal	2 families	20	5	—	25
Flecked	F 5/3/1	2	46	2	50
	2	—	7	1	8
	3	2	18	—	20
	4	2	21	1	24
	5	5	71	2	78
	9	1	15*	—	16
	2 families	—	41	—	41
	Total	12	219	6	237
	Percentage	5·1	92·4	2·5	100

* One flecked plant (No. F 5/3/9/14), description of which will be found in the text, changed vegetatively to normal and was evidently a mosaic of normal and flecked.

TABLE III.

Data collected in the pedigree culture of flecked in 1928.

Mother plant	No. of families	+	fl	"Fringed"	"White"	Mosaic* F. and N.	Mosaic* F. and Fr.	Mosaic* F., Fr. and N.	Total
Normal	6	111	—	—	—	—	—	—	111
	14	208	87	—	—	—	—	—	295
	1	87	28	—	—	—	2	—	117
	1	54	20	—	2	—	1	—	77
	1	47	15	—	—	1	1	—	64
	Total	396	150	—	2	1	4	—	553
Flecked	51	—	1265	—	—	—	—	—	1265
	48	112	1722	—	—	—	—	—	1834
	9	—	408	—	15	—	—	—	423
	20	42	707	—	25	—	—	—	774
	2	—	57	—	—	2	—	—	59
	1	—	23	—	—	—	1	—	24
	2	—	42	—	—	—	—	3	45
	1	—	59	2	1	—	—	—	62
	1	—	36	—	1	—	1	—	38
	2	3	69	2	—	—	—	—	74
	7	19	379	—	—	—	10	—	408
	1	3	75	—	—	1	—	—	79
	1	2	52	1	2	—	—	—	57
	2	6	112	—	2	2	—	—	122
	1	1	18	1	—	1	—	—	21
	3	15	216	3	—	—	4	—	238
	1	6	47	—	2	1	2	—	58
	Total	209	5287	9	48	7	18	3	5581
	F 5/3/9/14—*F*	2	38	—	—	—	—	—	40
	—*S*	20	10	—	—	—	—	—	30

* F. = flecked, Fr. = "fringed," N. = normal.

individuals. The normal mutants, which appeared in the preceding generation, were revealed to be heterozygous for flecked, and the flecked families reproduced such normal mutants. In 1928[1] an extensive examination on the fifth generation was made, and the data collected are given in Table III.

The progenies of 177 plants, including 154 flecked and 23 normal flowers, were examined. Repeating the manifold variability, which was observed in the foregoing generations, flecked showed further variation, producing some "fringed" flowers in both seminal and vegetative ways. The "fringed" is a unique flower colour form, the details of which will be described later.

White variation.

Table II contains some white variants, which are entirely free from variegation throughout their plant life. During the flowering time, the writer again and again examined the flowers of the plants, which were recorded as whites, but he still found some pure whites. The whites shown in Table II are only those which were verified by daily observation. These white variants did not breed true to type in the subsequent generation, but gave nearly the same results as those obtained in the offspring of the ordinary flecked plants. In all, six white plants were used for examination, and the data thus obtained consisted of 3 normal, 137 flecked, 1 "fringed" and 4 "white." The data show the fact that the white variants appearing in the flecked families are not due to a genic change, but to a temporary variation.

Sometimes also branches bearing white flowers were observed on the flecked plants. After careful examination, such flowers were selfed, and progeny consisting of 6 normal and 61 flecked plants was obtained. This is practically the same as expected in the offspring of flecked. Therefore the white variation occurring vegetatively is also due to a fluctuating variation.

Normal mutants.

The appearance of the normal self-coloured mutants in the progeny of flecked is a recurrent phenomenon, and it is due to the inconstancy in the property of the gene, which is contained in the flecked flower. The total data, which are available from Table III, include 209 normal individuals among 5581, the proportion of the normal mutants being 3·7 per cent. From the total data collected through three generations the

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[1] On account of a trip abroad, the writer left the observations to Mr Tabuchi, who carefully recorded the flowers.

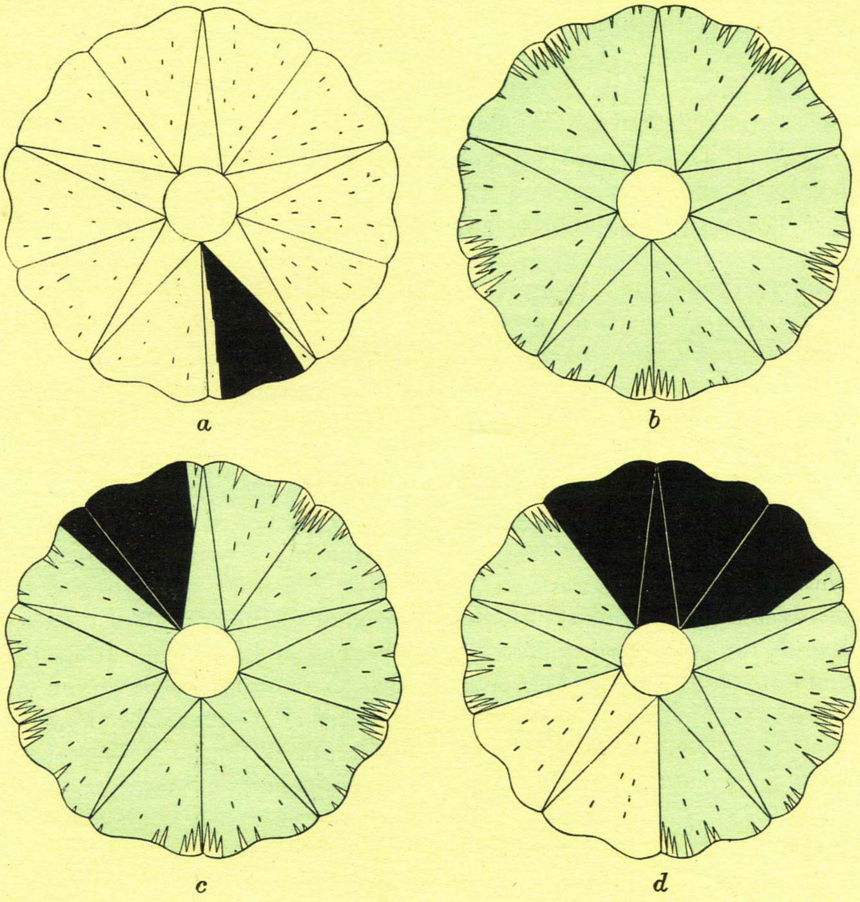
proportion is 3·8 per cent. (227 normals among 6002). In all, ten normal mutants were examined for their genotype; nine of them proved to be heterozygous for normal, and one of them was homozygous for normal. Owing to the constancy of the normal gene derived from flecked, a pure normal mutant pedigree was constructed. The mutable flecked originally might have appeared by a sporadic mutation from normal. Flecked may change into stable normal. If so the flecked mutation is of the nature of a reversion, returning to the prototype. When the normal mutant gene, however, is also mutable, as was observed in the other plants such as *Celosia* and *Plantago*, it cannot be regarded as reversion, because the two normals (one, prototype; and the other, inconstant normal mutant), even though apparently the same, are manifested by the different genes, one being non-mutable normal, and the other, mutable normal.

Mutating stages.

The mutating aspect of "yellow-inconstant" (Imai, 1930) brought the writer to the conclusion that the frequency of recurring somatic mutations varies in the different stages; that is, the mutability is high in the embryonic development, low in the post-embryonic somatogenesis, and again high in an ending stage of cell generations of leaves. This fact shows the different responsibility of the mutability of the gene "yellow-inconstant" for the different stages of somatogenesis, which works as an environment. In flecked, somatic mutations also appear in the early stage of plant cycle, some seedlings being characterised by self-coloured areas or stripes. The anthocyanin pigment, if present, is manifested very clearly on the hypocotyls. The self-coloured areas of the mosaic-flecked plants can generally be traced back to an embryonic origin, as in the case of "yellow-inconstant." The stripes or flecks appearing on corollas may be considered as due either to the propagation of the mutated cells or to the physiological differentiation of the character. The frequency of the self-coloured mutants in the flecked pedigree is 3·8 per cent. The corresponding green mutants have a proportion of 1·9 per cent. in the "yellow-inconstant" pedigree. In the latter, fine green spots appearing on leaves are evidently due to the propagation of the mutated cells. A corresponding mutation may also occur in the tissues of the flower, though actually the green cannot be visible. This relation suggests that the flecked characteristic of the corollas is due to somatic mutation, but the writer refrains from drawing any conclusion at present. Although the flecked corollas sometimes have rather broad stripes of an isolated distribution (Text-fig. 5 *a*), the flecks are generally fine.

Periclinal forms.

In "yellow-inconstant," the heterogeneous composition of bud-variations can be determined by the colour of plastids contained in the tissues, whereas, in flecked, one is handicapped by the limited distribution of the anthocyanin pigment in the tissues. For the epidermis may be coloured only in the corollas, and the sub-epidermal cells only in the stems, the other tissues being free from anthocyanin pigment.



Text-fig. 5. Variations exhibited in flecked flowers. Explanation in the text.

In flecked, two temporary types besides "white" are to be observed, so far as the writer's investigation is concerned. One of the two types has self-coloured stems and dilute flowers with fringed margins; the other has green stems and self-coloured flowers of the ordinary tones. The dilute flowers are also flecked and characterised by the presence of small white areas at the margin of the corollas (Text-fig. 5 *b*). Such a form appears either as an individual, in which all flowers are characterised by this pattern, or as a bud-variation, including cases in which the "fringed" character occurs in a stripe on the corolla. The "fringed" pattern is invariably accompanied by the dilution of the flower colour and by a self-coloured stem. The offspring of three "fringed" plants contained 38 normal and 12 flecked, but no "fringed." The "fringed" branches occurring on flecked gave analogous data consisting of 29 normal and 9 flecked. The "fringed" flowers thus making their appearance were heterozygous for flecked and normal (self-coloured), the genetic aspect of "fringed" being quite the same as the self-coloured flowers of the coloured stems obtained in the flecked pedigree. Therefore, the sub-epidermal region of "fringed" became automatically heterozygous for a mutant gene which is normal. The reason why the "fringed" flowers appear on the fully coloured stems may be due to the fact that the plant is composed of heterogeneous tissues with mutant sub-epidermal regions, the epidermis remaining unchanged. The flower colour lies in the epidermal cells; therefore the flower in this case may be dilute with a "fringed" margin through the influence of the mutant sub-epidermal region, which is colourless but genetically self-coloured. The "fringed" flower gives, though not frequently, self-coloured flowers or "fringed" ones with self-coloured areas (Text-fig. 5 *c*). The self-coloured region is considered to be produced by the extrusion of the inner mutant tissues, leaving the prototypic epidermis. A flower mosaic for flecked, "fringed" and self-coloured parts (Text-fig. 5 *d*), though its occurrence is very rare, represents three possible conditions of flecked. The occurrence of small white parts, by which the flower is fringed, may be due to the fact that these marginal parts of the corolla are presumably constructed by the propagation of the epidermal cells, if a tentative suggestion is allowed at present. In chimaerical plants, *e.g.* in the white-over-green periclinal, both longitudinal sides of leaves are generally fringed with broad white regions containing mesophyll as well as veins. This suggests that the longitudinal margins of the leaves are formed solely by the propagation of the epidermal cells, and an analogous development may possibly be the case in the ontogeny of the corolla. The stem colour, however, lies in the sub-epidermal cells, therefore the stem of "fringed" is self-coloured, due to the manifestation of the mutant character.

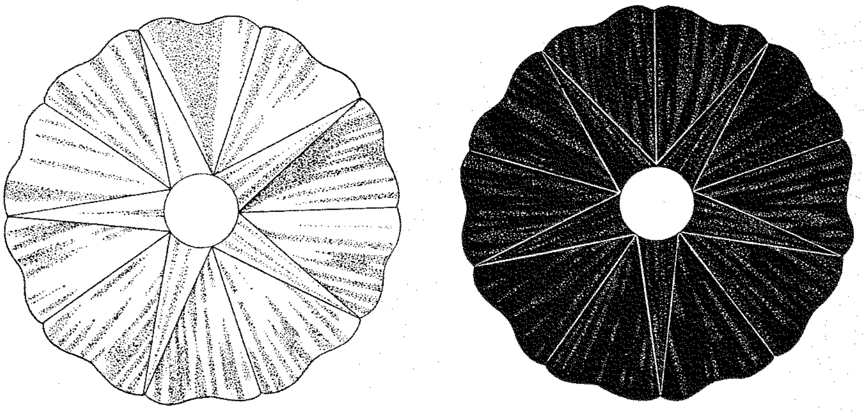
Self-coloured flowers were rarely borne on the green stems. This is another temporary form, occurring in the flecked pedigree. On selfing two such plants, the writer obtained 1 normal and 48 flecked, but no individuals like the mother plants. Therefore, the sub-epidermal region of the variants is genetically unchanged, remaining prototypic flecked, but their epidermis is altered by mutation of flecked to normal, resulting in the self-coloured flowers by the manifestation of the mutant epidermis. The stem, however, remains green because its sub-epidermal region is unchanged.

We may therefore conclude that the two temporary forms, one having coloured stems and dilute flowers with fringed margins, and the other having green stems and normal flowers, both rarely occurring in the flecked pedigree, are periclinal chimaeras. Hence the origin of these temporary plants is somatic, being invariably due to vegetative mutation occurring in an early stage of the development of flecked plants. In "yellow-inconstant" sometimes homogeneous mutant green branches were observed, and this allows the possibility of the somatic origin of green mutants. This argument is applied to the origin of some self-coloured mutants in the flecked pedigree. The majority of the self-coloured mutants, however, may originate through mutation in the mother plants.

DUSKISH PEDIGREE.

Variability of duskish.

The duskish flower has at times very fine normal-coloured spots or flecks on the otherwise duskish background (Plate V, fig. 7). Some-



Text-fig. 6. Two "ruled" flowers, showing variability.

times, however, it blooms into a variegated corolla on the same background, or again into a self-coloured flower by the extension of the normal colour. The variegation on the duskish flowers varies to a considerable extent (Text-fig. 6). Some plants bear flowers varying in the gradation of variegation as well as plain flowers, sometimes even self-coloured ones. The variegation occurs in fine stripes of dotted constitution or of solid broad areas. In the writer's investigation on the variation of duskish, he broadly classified it into two forms; one, "plain" duskish, and the other, "ruled" duskish; the former flowers as "plain" duskish, whereas the latter flowers sometimes or almost invariably as "ruled" duskish.

Inbreeding experiments.

The original plant, No. 361 *a*, with which the present experiments were made, was "plain" duskish. Selfing the plant gave a total of 109 individuals in the subsequent generation with the numerical contents as shown in Table IV.

TABLE IV.

Data collected in the pedigree culture of duskish in 1926.

+	"Ruled"	"Plain"	Exceptional	Total
2	1	105	1	109

The offspring of "plain" duskish contained one exceptional plant with white flowers. The genetic significance of this mutant will be stated later. The subsequent generation was observed, containing progenies of 40 plants, or 37 "plain," 1 "ruled" and 2 normal. The data thus obtained are shown in Table V.

The total of "plain" duskish families contains 1·1 per cent. of normal, 5·4 per cent. of "ruled" and 93·5 per cent. of "plain," whereas the "ruled" duskish family includes 4·5 per cent. of normal, 68·2 per cent. of "ruled," and 27·3 per cent. of "plain." The difference is so remarkable that it seems to be due to a genic diversity between the two classes. From the proportions in the "ruled" family one might suppose that there occurred a simple Mendelian segregation. This, however, was not the case, which became clear when the fourth generation had been observed. In Table VI are shown the data collected in 1929[1].

The behaviour of the variability is much complicated, owing to an irregular distribution in the frequency of the segregates.

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[1] In 1928 the pedigree culture of duskish was not made, owing to the writer's absence.

TABLE V.

Data collected in the pedigree culture of duskish in 1927.

Mother plant	Family no.	+	"Ruled"	"Plain"	Total
Normal	23	24	0	9	33
	41	50	0	18	68
	Total	74	0	27	101
	Percentage	73·3	0	26·7	100
"Ruled"	33	3	45	18	66
	Percentage	4·5	68·2	27·3	100
"Plain"	17 families	0	0	348	348
	5	0	1	116	117
	8	0	6	11	17
	14	0	1	30	31
	16	0	1	8	9
	17	0	1	29	30
	27	0	1	25	26
	28	0	1	27	28
	31	0	1	25	26
	32	0	2	31	33
	34	0	1	27	28
	4 families	6	0	132	138
	4	1	11	52	64
	7	1	26	44	71
	25	2	1	87	90
	26	1	1	18	20
	29	1	5	68	74
	39	1	3	18	22
	Total	13	63	1096	1172
	Percentage	1·1	5·4	93·5	100

TABLE VI.

Data collected in the pedigree culture in 1929.

Pedigree	No. of families		+	"Ruled"	"Plain"	Total
"Ruled"—normal	3		45	6	6	57
		Percentage	78·9	10·5	10·5	99·9
"Ruled"—"ruled"	5		16	17	35	68
		Percentage	23·5	25·0	51·5	100
"Ruled"—"plain"	1		0	2	14	16
		Percentage	0	12·5	87·5	100
"Plain"—"ruled"	19		69	91	236	396
		Percentage	17·4	23·0	59·6	100
"Plain"—"plain"	15		6	38	295	339
		Percentage	1·8	11·2	87·0	100

"Ruled" variants.

In Table VI, the proportion of normal, "ruled" and "plain" in the total "plain" families (from both "ruled" and "plain" grandmother plants) is 1·7 per cent., 11·3 per cent. and 87·0 per cent. respectively. This is practically the same as the corresponding case presented in the preceding generation (see Table V). The proportion in the "ruled" families, however, is 18·3 per cent. of normal, 23·3 per cent. of "ruled" and 58·4 per cent. of "plain." The production of normal is very great compared with that in the "plain" families. An inspection of the contents of the "ruled" families reveals the fact that the proportional difference in the segregating forms is remarkable. Under such circumstances, we cannot draw a final conclusion as to the precise nature of the variability of duskish, especially in relation to the "ruled" variant. But it is clear that: (1) "plain" gives sometimes (about 11 per cent.) "ruled" variants besides normals in its progeny; (2) "ruled" variants give a high proportion of "ruled" as well as a high proportion of normals; (3) the genetics of "ruled" variants is not simple.

Normal mutants.

Normals frequently appeared in the duskish families. Such normals proved to be heterozygotes. Tables IV and V contain the data showing this fact, and other similar results will be found in Table VII.

TABLE VII.

Offspring of normal mutants occurring in the duskish pedigree.

Family no.	+	"Ruled"	"Plain"	Total
12—1	9	1	1	11
13—1	29	1	7	37
29—1	6	4	2	12
Total	44	6	10	60
Percentage	73·3	10·0	16·7	100

In all, eight normal mutants were tested for their genotype and found to be heterozygous for duskish. The total progenies contain 163 normal, 12 "ruled" and 43 "plain," or 74·8 per cent. of normal, 5·5 per cent. of "ruled" and 19·7 per cent. of "plain." The duskish segregates occurred perfectly in accordance with a recessive ratio, their contents, however, being complicated by the irregular distribution of the "ruled" individuals. In Table VIII is collected the progeny of family No. 23, the original plant of which appeared as a normal mutant in the duskish pedigree.

Here no "ruled" duskish appeared. Owing to the small number tested, homozygous families for normality were not observed.

Rayed mutation.

In the direct progeny of No. 361 *a*, one exceptional plant, which was marked with white corollas, appeared among a total of 109, as shown in Table IV. The offspring of this variant were examined, and the results thus obtained proved that its appearance was due to a mutation. A statement on this mutation was presented by Imai and Tabuchi (1929). At that time the writers regarded the white mutant as a dominant white, named "White-4," because of its dominant aspect to duskish, from which the so-called "White-4" appeared. Crossing experiments with this mutant strain to the various strains, however, revealed that the white characteristic is due to the gene Rayed working on the duskish component, *i.e.* Rayed results in a white flower with coloured tube. Therefore the name "White-4" must be abandoned. From the genetic constitution of the white mutant, it can be understood why it has coloured stems and

TABLE VIII.

Later offspring of normal mutants.

Mother plant	Family no.	+	"Ruled"	"Plain"	Total
Normal	23—2	13	0	7	20
	—4	12	0	4	16
	—5	13	0	4	17
	—6	13	0	6	19
	Total	51	0	21	72
	Percentage	70·8	0	29·2	100
"Plain"	23—1	1	0	8	9
	—3	0	0	18	18
	Total	1	0	26	27
	Percentage	3·7	0	96·3	100

TABLE IX.

Data collected in the Rayed mutant pedigree.

Mother plant	Family no.	**Ry**	"Ruled" **Ry**	"Plain" **Ry**	+	"Ruled"	"Plain"	Total
Rayed duskish	4 families	0	0	364	0	0	0	364
	9	0	1	60	0	0	0	61
	Total	0	1	424	0	0	0	425
Rayed duskish	5 families	0	0	75	0	0	30	105
	2	0	0	38	0	2	15	55
	4	0	0	59	0	1	20	80
	5	0	0	72	1	0	26	99
	15	0	0	27	0	1	10	38
	16	0	0	62	1	0	15	78
	17	0	0	22	1	0	7	30
	20	0	0	44	1	0	15	60
	22	0	1	28	1	0	9	39
	Total	0	1	427	5	4	147	584
Duskish	4 families	0	0	0	0	0	147	147
	14	0	0	0	4	0	73	77
	19	0	0	0	1	0	43	44
	21	0	0	0	1	0	10	11
	Total	0	0	0	6	0	273	279

coloured tubes notwithstanding that it has white corollas. The rays of the corollas, however, are more or less tinged, especially on the back surface. As stated elsewhere, the progeny of the Rayed mutant consists of 25 individuals, among which 19 are duskish Rayed and 6 are duskish, indicating that the mutation brought about a plant heterozygous for a dominant Rayed gene. In 1927 the subsequent generation was observed, and the data gathered are indicated in Table IX.

The results prove the simple dominant nature of Rayed. The appearance of "ruled" duskish Rayed is due to the variable manifestation of the gene duskish.

SUMMARY.

1. The behaviour of 21 genes affecting the flower colour of *Pharbitis Nil* are described; namely, white-1 (**w1**), white-2 (**w2**), white-3 (**w3**) for white flowers, purple (**pr**), magenta (**mg**), dusky (**dy**), duskish (**dk**) for flower hues, intense (**i**), light-1 (**lt1**), light-2 (**lt2**), Dilute (**D**), tinged (**tg**) for flower tones, and speckled (**sp**), speckled-reduced (**sp-r**), faded (**fd**), smeary (**fd**^{s}), Rayed (**Ry**), flecked (**fl**), Lined (**Ln**), striated (**sa**), Blizzard-1 (**Bz1**). Of these genes, four are due to dominant variations. Faded and smeary are multiple allelomorphs of normal.

2. Two mutable genes, flecked and duskish, are closely studied. Flecked shows manifold variations. Sometimes normal self-coloured flowers with coloured stems appear as individual variants or bud-sports in the flecked pedigree. The occurrence of such normals is due to the mutation of the gene flecked to normal. The frequency of the occurrence of the normal mutants is 3·8 per cent. (227 normals among 6002).

3. A few "white" flowers may appear in the flecked pedigree, both as seminal and vegetative variations, but their manifestation is only temporary, fluctuating as an extreme variation of flecked. The majority of the offspring of the "white" flowers, therefore, are composed of flecked.

4. Two temporary types, besides "white," are observable in the flecked pedigree; the one has self-coloured stems and flecked, dilute-coloured flowers with fringed margins, the other has green stems and self-coloured flowers of an ordinary tone. The former type appears either as an entire plant or as a portion of a plant body. The "fringed" flower is considered to contain the mutant, sub-epidermal tissue, the epidermis remaining unchanged. The dilution of the flower colour is due to the effect of the mutant gene contained in the sub-epidermal tissue underlying genotypically colourless epidermis, because the anthocyanin

pigment is contained in the cells of epidermis in the flower. The stems of "fringed" are self-coloured, because the anthocyanin pigment is contained in their sub-epidermal region, which consists of self-coloured mutant cells. Therefore, the "fringed" type is a periclinal with a sub-epidermal mutant tissue. The breeding experiments prove the chimaerical nature of "fringed."

5. Another temporary form, green-stemmed self-coloured flower, is regarded as a periclinal with mutant epidermis and prototypic sub-epidermal cells. In this case, the flower is fully coloured, on account of its mutant epidermis; and the stem is green, on account of its unchanged, flecked sub-epidermal region. The majority of the offspring of this type, therefore, revert to flecked.

6. Duskish gives also normal plants in its pedigree, the appearance of normals being due to mutation. The frequency of such mutants is about 1 per cent. in the ordinary ("plain") pedigree. In addition to normal mutants, duskish gives at times "ruled" variants, in which the flowers are variegated. The amount of variegation varies to a marked degree. The "ruled" character is complicated, giving normal mutants as well as "ruled" variants, both in rather a high proportion.

7. Rayed mutation occurred in a duskish family. The mutant plant had coloured stems and bore white flowers with coloured tubes. Working on duskish the gene Rayed produces a white flower. Owing to the dominance of Rayed to normal, the mutant white flower is transmitted as a dominant character in the duskish family.

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EXPLANATION OF PLATE V.

(Each reduced to about two-thirds of the actual size.)

Fig. 1. Normal or standard.
Fig. 2. Intense.
Fig. 3. Purple intense.
Fig. 4. Magenta intense.
Fig. 5. Purple magenta intense.
Fig. 6. Dusky intense.
Fig. 7. Purple magenta duskish intense.
Fig. 8. Light-1.
Fig. 9. Dilute.
Fig. 10. Tinged.
Fig. 11. Speckled.
Fig. 12. Speckled-reduced.
Fig. 13. Rayed.
Fig. 14. Speckled Rayed (strain, no. 78).
Fig. 15. Smeary (margined).
Fig. 16. Blizzard-1.
Fig. 17. Striated.
Fig. 18. Lined.

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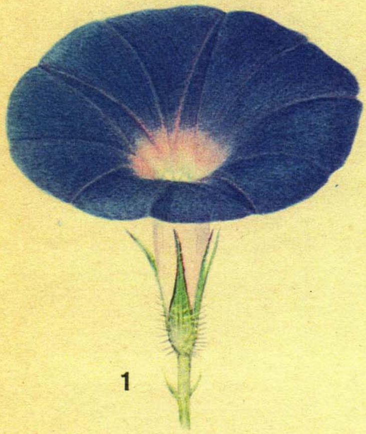

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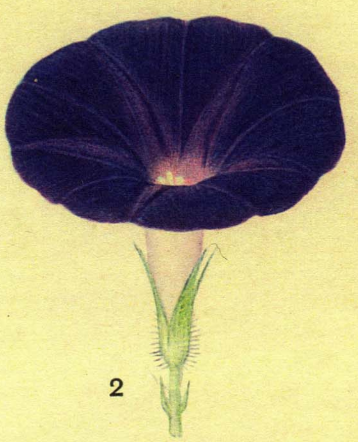

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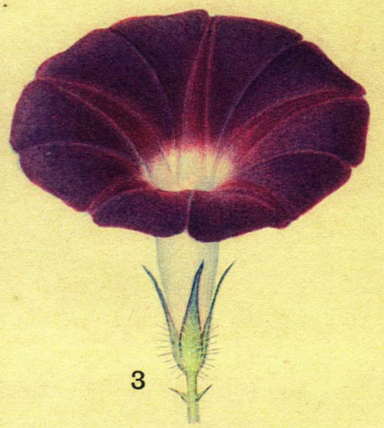

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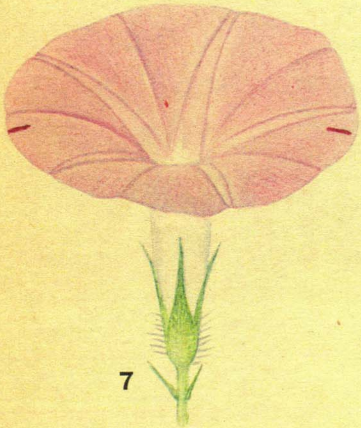

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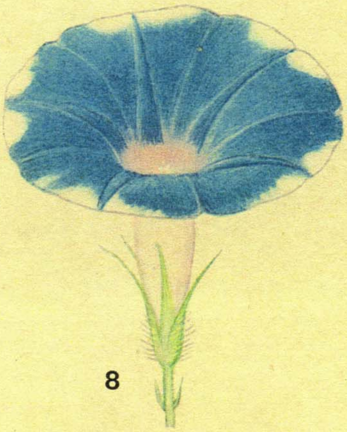

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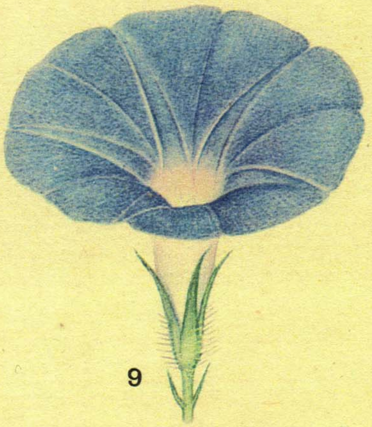

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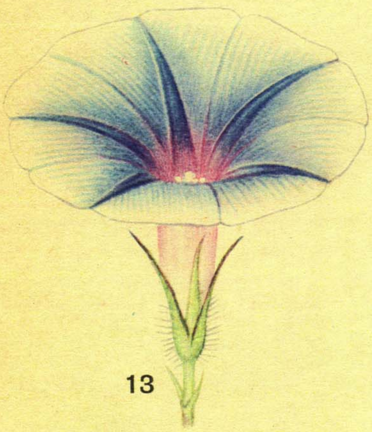

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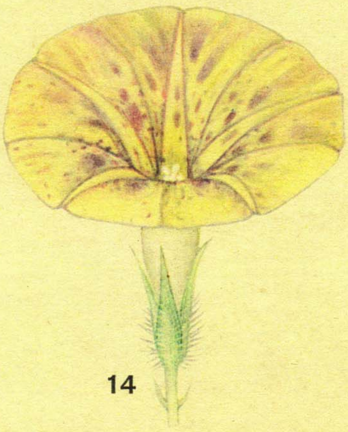

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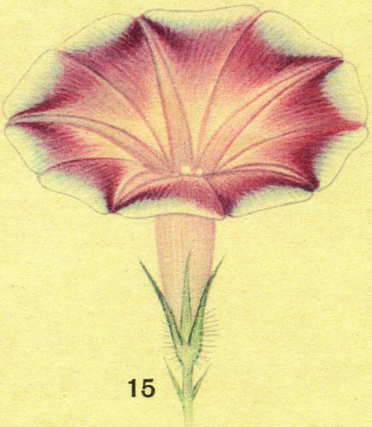

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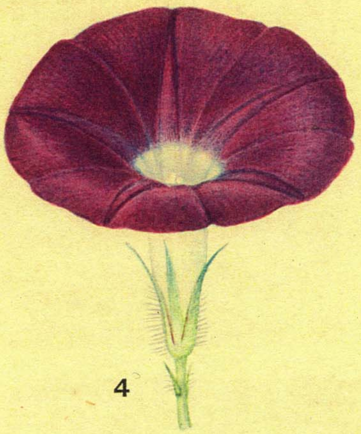
PLATE V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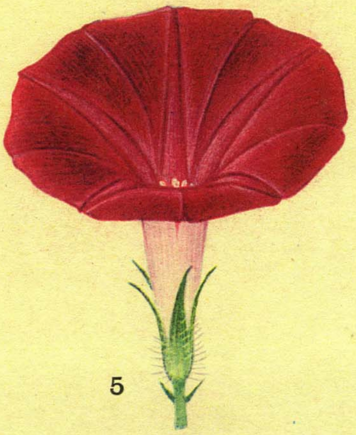
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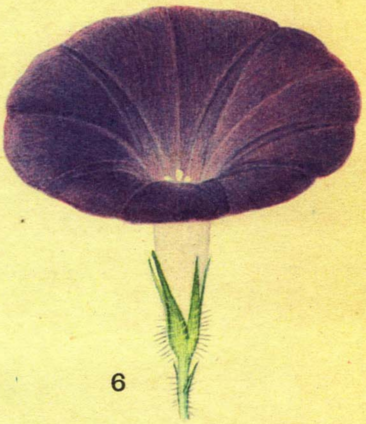
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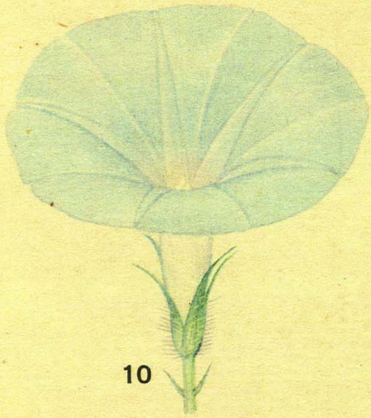
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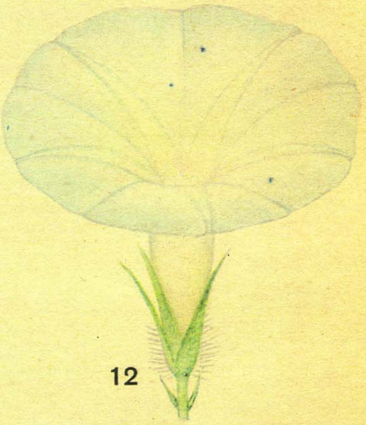
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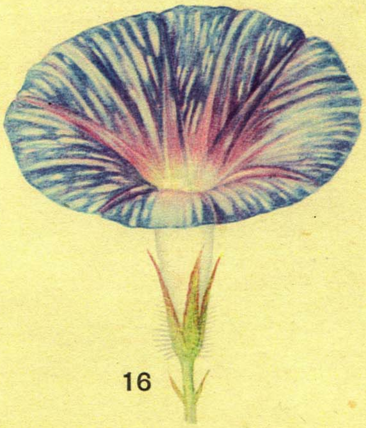
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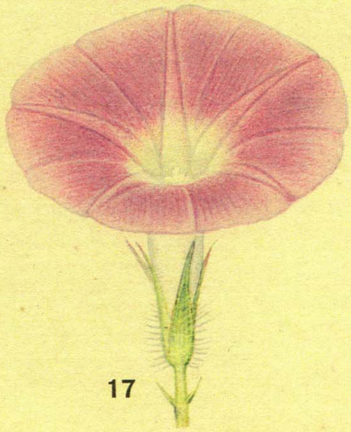
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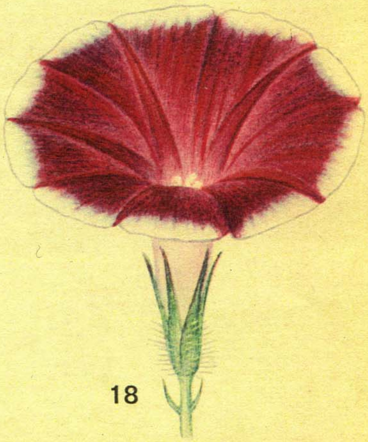
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