Setulæ stellatæ hypharum 40–70 μ longæ. Sporæ ovoideæ hyalinæ, 7–8 $\mu \times 5\ \mu$.

On the Rôle of the Factors C and R in the Production of the Flower Colours in *Pharbitis Nil.*

By

Tokio Hagiwara.

In *Pharbitis Nil* (Japanese Morning Glory) white-flowered individuals are commonly green-stemmed, but there are some white-flowered ones with coloured stems. The former have generally coloured tubes, though not always, while in the latter the tubes are always light yellow so far as his knowledge goes. These two white strains behave mostly as a simple Mendelian recessive to coloured flowers. The colour production in corolla was studied by the author by crossing together these white strains and also by crossing these white strains with the normal coloured one. The pedigree No. 106 bears white flower and coloured stems, while another No. 5 is characterized by the white flower with coloured tubes and green stems.

The purpose of the present paper is to consider, with especial reference to the pigmentation of stems and tubes, only the results of the crossing of No. 5 and No. 106 with normal coloured strains, and of the between these white strains themselves.

Experimental Results.

I. Colour flowered strain *versus* White flowered one with Coloured tubes and Green Stems.

The genetic relation between these two strains has been already studied by Miyazawa[1], Imai[2] and the author[3], and it was found that the white is recessive to the coloured. The author has many data to confirm this result, but only the result of two crossings between the white strain No. 5 and the coloured strains 51 A on one side and 9 A on the other will be described here.

All the F_1 plant from these crossings have produced coloured flowers with coloured tubes. The segregation in F_2 is as follows.

1) Journ. of Genetics, Vol. 8, 1918.
2) Bot. Mag., Tôkyô, Vol. 35, 1921. (Japanese)
3) Bot. Mag., Tôkyô, Vol. 37, 1923. (Japanese)

Table I

Cross	All with coloured tube		Total
	Colour-stemmed, coloured flower	Green-stemmed, white flowered	
5× 9 A	252	66	318
5×51 A	105	36	141
Totals	357	102	459
Deviation	±12.75		

The ratio of segregation is thus not very far from the simplest 3 : 1 expectation. The segregated whites are without exception colour-tubed and green stemmed, though some whites are coloured faint in tubes. There are neither whites with coloured stems nor any other kind of whites.

The F_3 data from the crossings, 5×9 A and 5×51 A are summed up in the following table.

Table II

Cross	Offspring which bred true to coloured-tube-and coloured flower	Offspring which segregated into colour flowered and colour-tubed white	Offspring which bred to colour tubed white flower	Total
5× 9 A	46	53 *	24	122
5×51 A	6	13	10	29
Total	51	66	34	151
Expected	37.75	75.50	37.73	
Deviation	13.73	9 50	3.75	
$\chi^2=6.53$		$P=0.0404$		

The goodness of fit is somewhat poor. This may be due perhaps to the fact that the F_2 plant self-fertilized have given in many cases but few seeds. On the whole, the actual numbers given in Table II are near to the expectation calculated on the basis of the ratio 1 : 2 : 1.

So it may be concluded that the white in this case behaves a simple Mendelian recessive to the coloured.

* See, Table III in which the segregates from these fiftythree families are tabled.

II. Colour-flowered strain *versus* Light yellow-tubed White flowered one with coloured stem.

The genetic study between these two strains was worked out by IMAI,[1] who has established the fact that this white strain is a simple Mendelian recessive to the normal coloured one. Through the crossing of the white-flowered strain No. 106 with the colour-flowered one No. 139, the author could confirm his results. The F_1 plant bearing coloured flower throw up the following segregates in F_2.

Table IV

Cross	Coloured flower and coloured stem		White flower and coloured stem		Total
	Colour-tubed	Light yellow-tubed	Colour-tubed	Light yellow-tubed	
106×139	47	—	—	16	63
Expectation	47.25	—	—	15.75	63.0

The coloured flower and the white flower similar to those borne by their parents were segregated out in the ratio 3:1. Neither light yellow-tubed, coloured flowers nor colour-tubed white flowers were segregated out in F_2.

The fifty F_2 families were raised for investigating the further generation. The summary of the results obtained in F_3 is as follows.

Table V

	Offspring which breeded true to coloured-flowers	Offspring which segregats into colour-flowered and colour-stemmed white flowers	Offspring which breeded to colour-stemmed white flowers	Total
Actual	16	20 *	14	50
Expected	12.50	25.00	12.50	50.0
Deviation	3.50	5.00	1.50	
$\chi^2=2.16$		$P=0.3447$		

The white-flowered offspring segregated out in F_3 are without exception colour-stemmed as in F_2, the tube being light yellow. The ratio

1) Bot. Mag., Tôkyô, Vol. 35, 1921.

* The segregates from these twenty families are ae tabled in Table VI.

of the family-numbers obtained by raising the F_3 plants is very near to the expectation 1 : 2 : 1.

From above we may conclude that white flower with coloured stem transmits to offspring both white corolla and coloured stem, which is a simple Mendelian recessive to the normal coloured flower. The white in this case has always light yellow tube.

III. Colour-tubed White strain with Green stem *versus* Light Yellow-tubed White one with Coloured Stems

The experiment done to elucidate the relation of the two white strains just mentioned is one of the most important, items to be reported in the present paper. Similar experiments have been done already by TAKEZAKI[1], MIYAZAWA[2], IMAI[3], and the author[4].

Among them TAKEZAKI's experiments refer simply to corolla, so it is not clear whether the two whites used in his crossing are identical with our whites. MIYAZAWA has made the crossing between the colour-tubed white and the light yellow-tubed one, and it may be supposed that the latter has coloured stem, though he has stated nothing in respect to this point. IMAI has done the crossing between the two white strains with green and coloured stem respectively, but he has given neither the results in F_3 nor any description about the tube-pigmentation.

As there exists the important relation between the colour of corolla on one side, and the pigmentation of tube and stem on the other, the author was concerned in the crossing between these two white strains, with especial reference to the interrelation between pigmentation of stem and tube and that of corolla. Many crossings between such two white strains were made and the study was contained till F_1, F_2, and sometimes further generation. All these crossings were in accordance to each other, so far as regards the production of coloured flowers. The results of the crossing is reported here in detail concerning 5×106. This crossing gave, as the F_1 plant, the colour-flowered plants with characters differing from those of either parent. In F_2 the segregation into four strains in accordance with the dihybrid ratio 9 : 3 : 3 : 1 was observed, although the deviation was somewhate large, since some white plants perished already as seedlings. The sum of the three white strains was much different from the expectation made on the basis 3 : 1, and it

1) Journ. of Japanese Breeder's Assoc. Vol. 1, 1916 (Japanese)
2) Japanese Journ. of Genetics, Vol. 2, 1923 (Japanese)
3) Bot. Mag., Tôkô, Vol. 25, 1921 (Japanese)
4) Bot. Mag., Tôkyô, Vol. 42, 1928

is very near to that on 9 : 7. (Table VII)

The production of coloured F_1 plants by the crossing of two white strains followed the F_2 segregation into coloured and white offspring were already found in many cases.

The coloured segregated out in F_2 have always coloured tubes and stems, of which some breed true, but others undergo the segregation in F_3 into colour and white in the ratio, either 3 : 1 or 9 : 7. The details of the raising of a further generation from thirty four coloured F_2 plants will be described in next lines.

Table VII

Cross	Coloured flower			White flower				Total
	Colour-stemmed		Green stemmed	Colour-stemmed		Green stemmed		
	Colour-tubed	Light yellow-tubed		Colour-tubed	Light yellow-tubed	Colour-tubed	Light yellow-tubed	
5×106 I	33	—	—	—	7	4	1	45
5×106 II	18	—	—	—	4	7	2	31
Actual	51	—	—	—	11	11	3	76
Expected (9:3:3.1)	42.75	—	—	—	14.25	14.25	4.75	76
Actual	51	—	—	—	25			—
Expected (9:7)	42.75	—	—	—	33.25			76

(a) Offspring of Colour-flowered Plant in F_2.

Sixteen families out of F_2 have undergone just the same segregation as is F_2 as shown in Table. The segregation of these families in total is as in Table IX.

Table IX.

	Colour-tubed, colour-flowered and coloured stem	Light yellow-tubed, white flower with coloured stem	White-flowered with green stem		Total
			Colour-tubed	Light yellow-tubed	
Total of sixteen families	369	742	159	39	709
Expected (9:3:3:1)	389.81	132.94	132.94	44.41	709.00
Deviation	29.81	9.06	26.06	5.31	
$\chi^2=8.590$		$P=0.0361$			

On the whole, the four forms appeared near to the proportion 9 : 3 : 3 : 1, but the deviation is comparatively large on account of a low linkage between two factors concerned. This linkage relation is to be explained in another paper which will describe one of coupling and six cases of repultion. The crossing over is 40-45% in case of repulsion. Seven families out of F_2 have segregated out only the lighty yellow-tubed whites with coloured stems. No. 28 has segregated out so many whites that it is impossible to consider the segregation to be in accordance with the ratio 3 : 1. It was hence omitted from these families, and the remaining six families were found to give the segregation, 207 coloureds and 68 whites, which is to the ratio 3 : 1. Eight families out of F_2 have segregated out the colour-tubed whites with green stems in the monohybrid fashion. In total the segregation which amounts to 300 coloureds and 94 whites accords almost with the expectation, coloured : white 295.50 : 98.50 on the basis of 3 : 1. Three families out of F_2 have bred true. No families were found, which have undergone in F_3 into coloureds and the light yellowtubed whites with green stem according to the 3 : 1 ratio.

(b) Offspring of Whites with Colour stem

Five families out of F_2 gave the segregation as shown in Table X.

Table X

Family No.	White with coloured stem	Light yellow-tubed white with green stem	Totals
6	56	21	77
15	42	11	53
17	31	15	46
23	54	12	66
38	47	19	65
Actual	230	78	308
Expected (3:1)	231.0	77.0	308.0

As shown in Table X, light yellow-tubed whites with green stem were segregated out in accordance with the ratio 3 . 1, but no colour-tubed whites with green stem were found. Two families out of F_2 bred true to colour-stemmed whites.

(c) Offspring of Colour-tubed White with Green Stem

Three families out of F_2 have segregated out colour-tubed whites and light yellow-tubed whites as shown in Table XI, which is near to the expectation 3 : 1.

Table XI

Family No.	Colour-tubed whith	Light yellow-tubed white	Total
21	35	9	44
2	43	5	48
29	37	16	53
Actual	115	30	145
Expected (3:1)	108.75	36.25	145.0

One family out of F_2 bred true to colour-tubed whites.

(d) Offspring of Light yellow-tubed White

Only one family of this white was inspected as to its offspring. Though only one family of the further generation was raised, we may be able to recognize the fact that this white breeds true always, as far shown in this crossing.

Discussion

The white strain with light yellow tube and green stem, obtained in F_2 of the crossing 5×106 is such having characters found niether in F_1 nor in the parents. This white is segregated out about 25% from the colour-tubed white, as well as from the colour-stemmed white, but not from the coloured flower. Hence this white behaves as recessive to either colour-tubed or colour-stemmed whites, in other words, it is hypostatic to any one white which appears in F_2 of this crossing. The genetic behaviour of this white and other two kinds of white will be shown clear by the inspection of Table XII made on the basis of the results obtained by the crossing 5×9 A, 5×51 A, 106×139, and 5×106. On this table one can see that F_2 segregation of the crossing between two white strains, 5×106 is complex in comparison to that of another crossing reported in this paper. Considering only the corolla of offspring derived from F_1 coloured plants their 56.25 percent is coloured against 43.75 percent white, that is according to the modified dihybrid ratio 9 : 7. Hence, as in the case of Sweet Pea and many other examples of flower-colour, this case may be interpreted in terms of the presence and absence of two factors.

Such two factors which are complementary for producing flower-colour were called *C* and *R* by Takezaki[1] and other investigators.

1) Journ. of Japanese Breeder's Assoc., Vol. I, 1916 (Japanese)

Table XII

Cross	F_1	F_2	F_3	
5× 9 A 5×51 A	Coloured flower 100%	75 %, Coloured flower	25 %, Coloured flower, ...100% 50 %, Coloured flower, ... 75%	White flower, 25% coloured tube
		25 %, White flower, coloured tube	.....White flower,..100% coloured tube	
106×139	Coloured flower 100%	75 %, Coloured flower	25 %, Coloured flower, ...100% 50 %, Coloured,..... 75% flower	White flower, 25% coloured stem
		25 %, White flower, coloured stem	.....White flower,..100% coloured stem	
5×106	Coloured flower 100%	56.25%. Colour flower	11.11%, Coloured,.....100% flower	
			22.22%, Coloured,..... 75% flower	White flower, 25% coloured tube
			22.22%, Coloured,..... 75% flower	White flower, 25% coloured stem
			44.44%, ...	Coloured flower, 56.25% White flower, coloured tube 18.75% White flower, coloured stem 18.75% White flower, light yellow tube 6.25%
		18.75%, White flower, coloured tube	25 %, White flower,..100% coloured tube 50 %, White flower,.. 75% coloured tube	White flower, 25% light yellow tube
		18.75%, White flower, coloured stem	25 %, White flower,..100% coloured tube 50 %, White flower,.. 75% coloured tube	White flower, 25% light yellow tube
		6.25%, White flower, lght yellow tube	.....White flower, light yellow tube 100%	

The coloured flower carries both these factors, but the white flower either lacks one of them or both. Since this crossing is between two white parents the one parent should be represented by *Cr*, and another by *cR*. As already described, one parent possesses coloured tubes and green stems, while another have light yellow tubes and coloured stems. Hence the question arises, whether the genetic formula of the colour-tubed whites is *cR* or *Cr*.

The author[2] have tried to decide this problem from the biochemical standpoint.

The flower-colour in this plant is produced by the interaction of

1) Bot. Mag., Tôkyô, Vol, 42, 1928.

at least two factors *C* and *R*, as in the case of some other plants. This fact that flower colours are not rarely due to anthocyanin pigment in cell sap, and also that the latter is produced by the reduction of chromogen consisting of flavone compounds, was made known by many chemists.

As the pigment of the flower-colour in the Morning Glory is anthocyanin and as so its production should be done at least by the two factors *C* and *R*, any one of the latter should be related either to chromogen or its reducing agent. It is well known that the flavone compounds are characterized by the production of yellow or orange colour when treated with alkalies, (WHELDALE[1], SHIBATA[2]), so that it is possible to detect their existence by exposing white flowers by ammonia vapour. The author has treated with ammonia the corolla of three white strains, and obtained the results as follows :—

	Flower types	Relative depth of yellow
I	Colour-tubed white	+ + +
II	Colour-stemmed white	+
III	Light yellow-tubed white with green stem	+

The results of this experiments gives the evidence that the white of the I type containes the flavone compounds most aboundantly, wheres in the other two white types only very little is present. So if we take *C* as the factor concerning such flavone compounds the white of I type i. e. colour-tubed whites with green stems should be represented by *Cr*, and consequently the other two white types by *cR* and *cr* respectively, i. e. light yellow tubed with coloured and light yellow-tubed with green stem.

The preceding four crossings may be represented therefore as follows :—

$5\times9A$ $5\times51A$	I type whites flower × Coloured flower, $Cr\times CR$
106×139	II type white flower × Coloured flower, $cR\times CR$
5×106	I type white flower × II type white flower, $Cr\times cR$

The genetical constitution of the F_1 plant of 5×106 the crossing between two white strains, should be *CcRr*, hence we see the following segregation in F_2.

	Genetic formula	Ratio	
Coloured plant	*CR*	9	9

1) The anthocyanin pigments of Plants. Cambridge, 1916.
2) Bot. Mag., Tôkyô, Vol. 29, 1915.

White plant { I Colour-tubed ..... *Cr* 3
II Colour-stemmed .. *cR* 3
III Light yellow-tubed. with green stem .. *cr* ..... 1 } 7

The genotypic constitution of the F_1 plants as was determined by the F_3 data, are shown in Table XIII.

Table. XIII.

Characters	Actual	Phenotypes	Genotypes	Ratio	Actual family numbers	Expected family numbers
Coloured flowre	33	*CR*	*CCRR*	1	3	2.8125
			CCRr	2	8	5.6250
			CcRR	2	6	5.6250
			CcRr	4	16	11.2500
Colour-stemmed white	7	*cR*	*ccRR*	1	2	2.8125
			ccRr	2	5	5.6250
Colour-tubed white	4	*Cr*	*CCrr*	1	1	2.8125
			Ccrr	2	3	5.6250
Light yellow-tubed white	1	*cr*	*ccrr*	1	1	2.8125

It may therefore be concluded that the factors *C* and *R* are responsible for producing flower-colours and that the factor *C* concerns the flavone compounds.

The Relationship between the Factors *C*, *R* and the Pigmentation of Tubes and Stems

Both the colour-flowered strain or *CR* and the colour-tubed white one or *Cr* are as far as noticed in this paper, colour-tubed ; the stem is coloured in the former, while it is green, in the latter. Both the white with coloured stems or *cR*, and that with green stem or *cr* have light yellow tubes, and the stem is colour in the former and green in the latter.

So we see that there is the exact correspondence between the pigmentation of tubes on one side and the factor *C*, and also between the factor *R* and that of stems on the other. The factor *C* not only concerns the production of flower colour, but also may take part in that of tube colour. There are some colour-flowered plants with light yellow tubes, hence the colour of tubes may be produced by the factor *C* and other factors. The factor *R* may concern the production of stem colour, besides being responsible for producing flower colour.

The author found the fact that there occurs a factor which be denoted as C^a, without which the factor C and R are unable to act in several organs of this plant. The plant lacking C^a, though procided with C and R, is light-yellow tubed[1] white with green stem and produces white seeds. The action of this factor may be considered as very interesting both from the standpoint of physiology and biochemistry. The genetic formula of this white group is c^aCR, c^aCr, c^acR and c^acr, as far his studies go. This will be the subject of one of his future papers.

Summary

Two complementary factors C and R for the production of flower colours have been found already by the former investigators. In plants lacking either any of them or both, the flower should be white in its corolla, though in the flower tubes or stems some white flowers may be coloured.

The author could ascertain through several crossing experiments the genetic relation of composition and the colouration as follows :—

Cr .....Colour-tubed white flower with green stem

cR.....Light yellow-tubed white flower with coloured stem

cr .....Light yellow-tubed white flower with green stem

The factor C is connected to some extent with the colour of folower-tubes, and R with that of stems.

The F_1 plant derived from the crossing between Cr white type and cR white type almost always segregates out in the next generation the coloured and the whites containing three types on the ratio 9 : 7. The results obtained by crossing the normal coloured flower with the third type white was not reported in this paper, and in this case the coloured F_1 plant was found to segregate the coloured and the whites on the same ratio as well as in the previous crossing, though the dihybrid ratio was somewhat disturbed on account of the occurrence of a coupling between the factor c and r.

There occurs another white group having yellow tubes and green stems just like the third type white, but producing white seeds. This is due to the lacking of the factor C^a, which activates C.

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1) The yellow colour of the tube is not undoubtedly due to anthocyanin, but probably to flavone compounds not yet rduced. It may appear without the action of C and R, and it seems to be not probabable that some special factor to this colour is present.

Appendix

Table. III.

Family No.	Colour with coloured tube and coloured stem	White whith coloured tube and green stem	Total
2	2	2	4
5	4	3	7
6	9	2	11
8	17	5	22
10	4	2	6
11	5	1	6
14	14	4	18
18	15	3	18
19	10	4	14
23	8	4	12
29	9	1	10
32	20	1	21
34	3	1	4
36	6	1	7
38	5	3	8
39	8	3	11
41	9	2	11
45	5	4	9
48	4	1	5
49	15	4	19
50	4	4	8
52	7	3	10
55	39	6	45
57	13	7	20
58	16	3	19
60	31	10	41
63	10	5	15
65	7	2	6
66	24	9	33
64	21	11	32
72	9	4	13
74	17	8	25
75	23	7	30
80	13	9	22
84	4	4	8
85	19	13	32
86	5	3	8
87	11	3	14
92	2	5	7
96	12	3	15
97	16	6	22
98	16	5	21
104	6	1	7
105	4	3	7
106	13	3	16
107	1	1	2
111	2	2	4
115	5	3	8
116	12	8	20
118	4	3	7
119	6	2	8
123	7	4	11
124	10	6	16
Actual	561	217	778
Expected (3:1)	583.5	194.5	778.0

Table. VI.

Family No.	Colour with coloured tube and coloured stem	White with light yellow-tube and colour-stem	Total
3	15	4	19
4	13	5	18
5	43	10	53
6	9	2	11
8	22	7	29
11	12	2	14
12	26	6	32
18	15	4	19
20	3	1	4
22	22	5	27
28	25	18	43
30	8	3	11
37	25	11	36
39	14	6	20
43	18	9	27
44	20	8	28
46	5	3	8
48	5	3	8
52	5	2	7
58	18	9	27
Actual	323	118	441
Expected (3:1)	330.75	110.25	441.0

Table. VIII.

Family No.	Colour-tubed colour with coloured stem	Colour-stemmed white with light yellow tube	Green-stemmed white		Total
			Coloured tube	Light yellow tube	
1	30	11	16	5	62
3	16	3	5	3	27
4	21	7	8	1	37
8	24	10	13	3	50
10	24	14	13	2	53
14	29	12	16	1	58
16	62	23	22	3	110
20	11	3	4	1	19
22	18	8	7	2	35
25	41	15	13	3	72
30	37	17	19	3	76
35	5	1	2	0	8
36	9	1	2	1	13
39	16	7	6	2	31
40	14	6	7	1	28
41	12	4	6	8	30
Total	369	142	159	39	709
2	16	—	4	—	20
5	50	—	16	—	66
7	38	—	10	—	48
26	47	—	10	—	57
32	29	—	9	—	38
34	86	—	26	—	112
45	17	—	12	—	29
46	17	—	7	—	24
Total	300		94	494	394

11	74	20	—	—	94
12	20	12	—	—	32
13	16	7	—	—	23
19	25	4	—	—	29
28[1]	53	44	—	—	97
37	24	10	—	—	34
44	48	15	—	—	63
Totals	207	68			275
18	20	—	—	—	20
31	8	—	—	—	8
42	41	—	—	—	41

1) The number of segregated whites is too large.

Nuntia ad Floram Japonicae V.

auctore

M. Honda

32) **Ranunculus yatsugatakensis,** HONDA et KUMAZAWA sp. nov.

Radix fibrosa, alba, elongata. Rhizoma erectum v. ascendens, breve. Caulis erectus v. ascendens v. reptans, simplex v. ramosus, gracilis, 10–30 cm elongatus, praeter summam glabrescens. Folia radicalia 2–4, longe petiolata, petiolis 3–10 cm longis, glabriusculis gracilibusque, laminis reniformibus, 5–10 mm longis, 10–15 mm latis, glabris v. puberulis, basi subtruncatis v. saepius cordatis, palmato-5–9-lobatis, lobo medio obovato-elliptico, acuto, lobis lateralibus angustioribus, omnibus margine integris. Folia caulina 2–3, remota, inferiora petiolata, petiolis 5–15 mm longis, basi dilatatis vaginiformibusque, laminis profunde 5-lobatis, lobis linearibus, 8–15 mm longis, 1–2 mm latis, superiora sessilia v. brevissime petiolata, 3-partita, partibus minoribus. Pedunculus longe nudus, pubescens, axillaris, solitarius. Flos 6–8 mm in diametro, flavus. Sepala 5, ovatoelliptica, concava, pilosula, 3–4 mm longa, flavo-viridula. Petala obovato-oblonga, 4–4.5 mm longa, glabra, aureo-flava. Stamina 2 mm longa, antheris flavis. Ovaria glabra. Achenia globoso-glomerata, capite 4 mm in diametro. Receptaculum brevissime pubescens.

Nom. Jap. Yatsugatake-kinpōge (nov.)

Hab.

Hondo: in monte Yatsugatake, prov. Shinano (M. KUMAZAWA, anno 1928).