A CYTOLOGICAL AND A GENETICAL STUDY OF PETUNIA V

THE INHERITANCE OF COLOR IN POLLEN¹

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While many investigators have studied the problem of the inheritance of color in reference to the corolla, the fruit, and the seed in a considerable number of plants, only a few students of color in floral parts have made more than a casual reference to pollen color and its inheritance. The origin and distribution of color in the anther and in the pollen are to be considered in a later paper. At present we are concerned solely with the method of inheritance of pollen color.

In 1858, NAUDIN reported a series of experiments in hybridization which he had carried out during the summers of 1854, 1855 and 1856. His investigations were based on the two species, *Petunia violacea* and *Petunia nyctaginiiflora*. He noted that the former has a blue throat associated with "blue violet" pollen, while the latter has "pale yellow" pollen and a throat tinted with yellow and marked with delicate "brownish" veins. He found the first generation hybrids of these two species very uniform in all respects, and intermediate between the two parents. The second generation hybrids, even when derived from seeds of the same capsule, were markedly variable. Notwithstanding the fact that he made no attempt to prevent promiscuous pollinations in the field, his results are of great interest, even though many observations recorded are not in line with recent findings. He did not report observations on any F_2 family which consisted of less than 10 mature plants; and because of lack of space he brought to maturity not more than 40 plants in any one of his second generation families. In one family of 25 plants, 8 bore yellow pollen and 17 gray or blue pollen. Evidently because colored flowers were much more abundant in his cultures than white flowers, he frequently made the statement that *Petunia violacea* produces a much stronger effect upon the hybrid progeny than does *P. nyctaginiiflora*.

Some F_2 plants could not be distinguished from *P. violacea* in regard to morphological characters as well as to color of corolla and of pollen; while a lesser number of plants resembled in all respects, including yellow pollen, *P. nyctaginiiflora*. Still other plants bore flowers of various shades of color, ranging from that of the colored parent to that of the white parent; but

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always the plants with blue pollen were in excess of those with yellow pollen. In some the limb of the corolla was almost white, the throat purple and the pollen gray blue. He mentions especially two plants of an F_2 family which bore lilac-tinted corollas, one having yellow pollen and the other gray-blue pollen. At this early date, then, the fact that pollen color and color of corolla limb are inherited independently in certain hybrids of *Petunia* was clearly demonstrated, though not asserted, by NAUDIN. In many of his later papers, 1863, 1865, et cetera, NAUDIN gave further data regarding the inheritance of color and other floral characteristics in *Petunia*, but he added practically nothing of value to his earlier observations in reference to the inheritance of pollen color.

So far as I have been able to determine, no other investigations regarding the genetics of pollen color in *Petunia* were reported until some sixty years later. In 1918 RASMUSON made a brief study of a cross between a petunia plant with flowers having white limbs, blue throats and blue pollen and a plant bearing flowers characterized by violet limbs, yellow throats and yellow pollen, the latter being used as the pollen parent. These plants were evidently chance variations of cultivated strains and, as is to be expected, the F_1 plants were variable. He decided that one parent at least must have been heterozygous. The F_1 plants fell into four groups according to the colors of limb, throat and pollen. From a consideration of but 42 plants which were distributed among these groups, RASMUSON concluded that blue anthers and blue pollen are dominant to yellow anthers and yellow pollen with a monohybrid segregation in the F_2 . He further concluded, from an analysis of less than 50 plants, that with blue anthers and blue pollen the throat is always blue; that yellow pollen and yellow anthers are always associated with yellow throat; and that the gene for deep flower color and the gene for blue pollen are inherited independently.

We find in our cultures very clean-cut evidence that in certain hybrids the inheritance of limb color is entirely independent of that of throat, or of pollen color. Evidently the factors determining throat color and those controlling the color of pollen in these hybrids are not located on the same chromosome or chromosomes as are those responsible for limb color. Throat and pollen color, however, are always linked with a certain percentage of crossing over, so that pollen of a given color is usually, but by no means always, associated with a throat of a definite color. The genetics of throat color in relation to color of limb and to color of pollen is to be discussed in a paper to be published in the near future, and will not be considered further at this time.

The only other reference which I find regarding the inheritance of pollen color in *Petunia* is the statement recently made by DALE (1931). He affirms, as had RASMUSON earlier, that "pollen color in diploid petunias

shows monohybrid inheritance." The results of our observations, based both on species crosses and on crosses with certain cultivated strains which are homozygous for flower color, are not in accord with such a conclusion. DALE does not give the origin of his diploid petunias. Under usual conditions certain F_2 and F_3 diploid segregates would be expected to show a monohybrid inheritance. The species crosses, as well as those of the cultivated strains of *Petunia* which we are growing, indicate very clearly, as will be shown later, that several factors are concerned in the inheritance of pollen color. In accordance with our analysis, but 4 genotypes in every 256 F_2 plants of the species cross would breed true when selfed and give a monohybrid ratio for pollen color when selfed or when crossed with the homozygous recessive. These would comprise the yellow pollen of *P. axillaris*, 2 shades of green and 1 shade of blue.

METHODS AND MATERIAL

In order to secure unity in a series of records taken over a term of years and by different individuals, one of the first essentials is that there be worked out, as the result of preliminary experimentation, a definite method of procedure to be followed by all. It is a common observation that the flowers of petunia change color as they age; but we find that no marked differences in pollen color can ordinarily be detected between anthesis and the fading of the flower. Notwithstanding, it seemed desirable that the determination of all pollen color should be made between certain limits of time following the dehiscence of the anthers. As a rule the pollen of diploid petunias is shed on the same day as that on which the flower opens. When pollen is to be collected, all flowers are cut from the plants in the late afternoon of the day previous to that on which pollen is to be taken. Any flowers present on the next day must of necessity be freshly opened. If for any reason it is not desirable to remove all flowers, pieces of light twine are tied to the peduncles of such flower buds as would normally open the next morning. The same pollen varies appreciably in color depending on whether it is dry or moist. Collections of pollen are, therefore, never made in the early morning, on "foggy days," or directly after rain. Thus the pollen used in this study was both *fresh* and *dry*.

All the color determinations are based on Ridgway's Color Standards and Color Nomenclature. In matching the pollen to the standard colors, we found it necessary to have a uniform and carefully worked-out plan if results were to be, in any degree, dependable. Even with close adherence to the best of plans, we do not believe that errors, in assigning pollen to the intergrading hues of color which lie between the more clearly defined spectrum colors, can be entirely eliminated. As already stated, moist pollen has not the same shade or tint as has dry pollen; the hue may vary

appreciably with variations in the amount or depth of the pollen that is being considered; a given mass of dry, fluffy pollen will match to, or appear identical with, various tints or shades of color depending on the color of the background on which it rests. For instance, different tints or shades of yellow may be assigned to the same bit of yellow pollen if the pollen when being matched to the Ridgway colors rests on a dark yellow, a light yellow, a yellowish green, a gray or other colored background. After various trials we decided that the most satisfactory method is to place a small mass of pollen on or near the edge of a glass slide, and to hold the slide from an inch to an inch and a half above, with the pollen mass just at the margin of, the block of color with which the pollen is being compared. A piece of white cardboard with a rectangular opening the size of a single color block is placed over the Ridgway color plate so as to eliminate the influence on the eye of all colors except the particular one with which comparison is being made.

Since the intensity of light varies not only on different days but also at different hours of the same day, and since variations in light intensity change the tones of certain shades of color as registered by the eye, it has been our practice for some years to take all color records by means of a daylight lamp especially constructed for color-metric work, and in a darkened room, thus securing a certain degree, at least, of uniformity of light. We obtained from the publishers separate pages of their color plates which were most used in our work. With the particular design of lamp which we have, the lighted surface being an inclined plane enclosed on three sides, the separate plates are much easier to manipulate when one is taking records than is the book with its stiff covers. The color plates are always placed in a definite position with regard to the center of the lighted surface in order that color determinations may be made under as nearly identical light conditions as possible.

In securing pollen, the corolla is cut away at a distance well beneath the anthers. By means of a needle or a spear the filaments are tapped causing the pollen to be shed on a glass slide placed just beneath the anthers. As the amount of pollen varies in the flowers of the different members of a hybrid family, it is sometimes necessary to use pollen from more than one flower of a given plant. It would be very simple to hold the recently dehiscent anthers, with their pollen still adhering, directly over the blocks of color; but since the color of the anther, which may not be the same tint as that of the pollen, would influence one's judgment regarding the exact tone of the pollen color, such a procedure is not feasible.

The problem of "color matching" is further complicated by the fact that one's judgment varies at different times, so that one may match the pollen of a given plant to a certain hue or shade on one day and the pol-

len of the same plant to another, perhaps closely related, hue or shade on the following day or week. In addition to this, there is the well known fact that the human eye is quickly fatigued as the result of close application. For this reason, we do not plan to determine color tints or shades for longer than an hour at a sitting. It has been our intention to adhere very closely to the method, as outlined above, for the determination of pollen colors. But even so we are sure that the records which follow do not in all cases exactly conform to the various hues of color as they really exist in the plant. While it is usually very clear as to which of the large groups of spectrum colors a mass of pollen under consideration belongs, it is, in certain instances, equally difficult to decide as to just which tint of the Ridgway colors certain samples of pollen correspond. The greatest difficulty which we have encountered is in distinguishing between greenish yellow and yellowish green tints. To illustrate, it is, as a rule, well-nigh impossible to decide whether a certain tone of pollen should be listed as Pale Green Yellow or as Pale Viridine Yellow. Yet if assigned to the former tint it would fall in the yellow group, if to the latter tint, in the green group. In our final summary, we have placed one-half of the plants assigned to these two colors in the yellow group and one-half in the green group. This seems to us the only feasible solution, since the decisions here are so largely a question of judgment, and since during the last ten years records of pollen color have been taken not only by the writer but also by three assistants. Fortunately these tints do not often occur in the pollen of the plants under observation and, therefore, would not materially affect the ratios when large numbers are being considered. While recognizing such possibilities of error, we still feel confident that the ratios as obtained for the large groups of spectrum colors would not be substantially changed were it possible to eliminate every vestige of error. Since different people do not see colors exactly alike, we have been guided, in assigning the individual shades or tints to the groups of spectrum colors, and in listing them within these groups, by "the relative proportions of two or more colors in any mixture" as given by Ridgway.

The record of observations and the discussions which follow are based on the color behavior of the pollen in two distinct lines of hybrids. One series of F_1 , F_2 , and F_3 plants, together with the backcrosses to the parents and the intercrosses between members of the same F_2 family, was derived from crossing *Petunia axillaris* (Lam.) BSP. (*P. nyctaginiflora* Juss.) with *Petunia violacea* Lindl. The second series of hybrids had its origin in crosses between *P. axillaris* and a large-flowered garden variety with rich deep-purple corolla and Turtle Green pollen. This cultivated strain is known in our cultures as population eight (P8). We have grown this strain of petunia since 1919 and it has continued to breed true to color of floral parts for ten

generations. Unfortunately it was attacked by mosaic and during the winter of 1933 was completely lost to our cultures. A certain lessening of vigor as the result of long inbreeding may have increased its susceptibility, and its lack of resistance, to disease. We are inbreeding certain segregates from its crosses with *P. axillaris* and also segregates from crosses between it and another strain of deep purple-flowered petunias. It is too early to report the results of these efforts to segregate out a plant of the same genotype, in regard to floral characteristics, as the original strain. Records have been taken of pollen color for more than 22,000 plants of these two series.

There is every reason to believe that the two species considered in this study are the same as those described by NAUDIN in 1858 and used in his experiments. We recently redescribed and traced the history of these two species (FERGUSON and OTTLEY 1932). Not until our paper had been sent to press did the name and description of *Petunia Parodii* (STEERE 1931) appear. It is evident when one compares STEERE's description and illustrations (Plates XXXII and XXXIII) of *P. Parodii* with *P. axillaris* (Lam.) BSP., as described and illustrated by us in 1932, that the entity is one and the same for the two names. The concept accepted by STEERE for *P. axillaris*, as judged from his illustrations and text, agrees markedly with the cultivated strain, which we raised, in 1932, to the dignity of a horticultural species, assigning to it the name *Petunia alba*. We have not succeeded in finding any statement by STEERE regarding the origin of the plants which he accepts as *P. axillaris*. This information is undoubtedly given in an earlier paper but, if so, it has been overlooked by the writer.

Petunia violacea Lindl. is known in our cultures as population nine (P9) and *Petunia axillaris* (Lam.) BSP. as population fourteen (P14). The former has Cadet Gray pollen, the latter Pinard Yellow. The color records for the series involving the two species² have all been made in a darkened room by means of the daylight lamp. The earlier records for the series in which P8 was one of the original parents were taken on sunny days in the greenhouse and in the field, always with the back of the recorder so placed as to secure as nearly as possible north light, and never with the flower in direct sunlight. During the last four years color records for both series have been taken in a darkened room, by means of the special lamp to which reference has already been made, and with the pollen mass on a glass slide as described earlier.

As stated in 1932, we did not succeed in securing plants of *Petunia violacea* Lindl. until late in the summer of 1930. This species is rather reluctantly self-fertile, having been grown at Kew Gardens, the source of our plants, for 16 years without setting seed. Some experimentation was neces-

² I take pleasure in giving most cordial recognition of the very efficient work of BARBARA HUNT, my special assistant in carrying out the investigations on this species cross.

sary before seeds were secured as the result of selfings, and a year elapsed before the species was established in our cultures in sufficient numbers to enable us to use it freely in the work of hybridization. The largest number of seeds yet obtained, in a given capsule, is 82, whereas capsules resulting from selfing *P. axillaris* may contain over 1000 seeds. Also the percentage of germination is low, rarely exceeding 50 percent, often considerably less. This refers to the F_1 and F_2 families of the crosses of P9 with P14 as well as to capsules resulting from selfing P9. For these reasons, the records of pollen colors for the crosses between P9 and P14 and later generations are much fewer in number than are those of the series of hybrids resulting from the various crosses involving P14 and P8. However, since *Petunia violacea* (P9) and *Petunia axillaris* (P14) have been universally accepted as the species which, through free intercrossing under cultivation, have given rise to the "garden petunias" of all countries, it seems desirable that the species crosses and their progeny should be considered before taking up the crosses involving the cultivated strain, even though the report does not cover so large a number of plants.

PETUNIA AXILLARIS (Lam.) BSP.(P14) $\times$ PETUNIA VIOLACEA LINDL. (P9)

As indicated in an earlier paper (BROOKS, WALSH and FERGUSON 1930), the pollen of P14, while very active on its own stigmas and to a lesser degree on those of its own sibs, is almost 100 percent inactive on the stigmas of all other species that we are growing and of all cultivated strains in our cultures not immediately related to it. For this reason, P9 is the pollen parent for all the F_1 plants which we have grown in this series. Backcrosses to both parents have been made with comparatively larger families resulting from the crosses between P14 and the F_1 than those resulting from crosses between the F_1 and P9.

In an attempt to explain and to correlate our observations on the various crosses involved we have assumed that 4 pairs of allelic factors are operative in the inheritance of color in the pollen of *Petunia*.

B is a factor for blue and is dominant; *b* gives very delicate tints of blue.

Y is a factor for yellow and acts as an incomplete dominant. The recessive, *y*, gives very light tints of yellow, and *Yy* is intermediate in its color reactions between *YY* and *yy*.

B and *Y* together produce green. Yellow color is possible only in the absence of *B* and likewise blue color can occur only in the absence of *Y*; but the dominant of the one prevails over the recessive of the other, as *B* over *y* and *Y* over *b*. The combination *by* gives very delicate shades of green.

D is a factor for gray and is an incomplete dominant, *Dd* being intermediate in its reactions between *DD* and *dd*. The recessive, *d*, gives white and acts only as a dilution factor. *D* cannot express itself as gray under any circumstances, and acts only to tone down or dull the other colors.

G is a factor for green and produces, phenotypically, a different tone of green than that given by the association of genes *B* and *Y*. It is a partial dominant; but *Gg* is only slightly weaker in its color reactions than *GG*. *G* is hypostatic to both *B* and *Y* and, therefore, the difference between *GG* and *Gg*, as agents of pigmentation, becomes apparent, only when both *B* and *Y* are absent. In the absence of *D*, *G* gives a greenish cast to the yellows and deepens the greens and the blues, but it cannot express itself as green except in the entire absence of both *B* and *Y*. The recessive, *g*, produces very pale green.

It follows that the various spectrum colors and the intergrading hues depend for their expression not only on the amount or dosage of each color factor but also on the reactions of these factors to one another. The factorial analysis, as above stated, is at best but a clumsy attempt to express the many and the more refined reactions that undoubtedly take place in petunia plants in the development and expression of color in floral parts.

Based on the assumptions as outlined above, the Cadet Gray pollen of *P. violacea* is represented by *BBDDGGyy*, the Pinard Yellow of *P. axillaris* by *bbddggYY*, and the Greenish Glauous-Blue pollen of the F_1 by *BbDd-GgYy*. Records have been taken of the pollen color of 5 families of first generation hybrids comprising 237 plants, and we have never observed a single instance of color other than that of Greenish Glauous-Blue which, as will be seen by reference to table 1, is a shade of green. This is not then a case of clean-cut dominance as affirmed by the writers referred to earlier. The F_1 pollen is not the color of that of either parent; rather it is a new color which may possibly be interpreted as intermediate between that of the two parents, although it actually contains neither orange nor yellow. Apparently the orange and yellow pigments of the Pinard Yellow (P14) have so reacted with the blue, green, and gray pigments of the Cadet Gray (P9) as to produce the grayish blue-green pollen of the F_1 . It is found that a considerable range of color occurs in the pollen of the second generation hybrids. The assumptions which we have stated provide for at least 29 shades or tones of color in the F_2 plants. These are distributed within three groups of spectrum colors — blue, green, yellow.

It is not to be expected that all these intergrading color tones would be detected, and in their relative frequency, without observing a very large number of plants; especially would such an expectation not be entertained when one considers the low percentage of seed germination in this series in connection with the fact that in practically 15 percent of these F_2 plants there was a complete absence of pollen. In some entire families the color records in this series are based on the second flower that appeared on a plant under normal conditions and, because of space limitations, the

plant was destroyed immediately following the taking of its records. These plants, recorded as producing no pollen, would have undoubtedly, in many cases, yielded pollen at some later time had we been able to furnish space for their continuance. With rare exceptions, their anthers were normal in size and structure but devoid of pollen. In addition, one must bear in mind that the conditions governing the action and interaction of the color factors, as assumed, are such that 5 tints of color would normally appear but once each in 256 plants, while one shade may appear 48 times. The other colors vary in the possibility of their appearance from 2 to 24 times in every 256 plants; and no F_2 family yet obtained in this cross has yielded one-fourth that number of mature plants. It was, therefore, not without surprise mingled with satisfaction that we found, on summarizing our data for these F_2 plants, a very close approximation to the theoretical results imposed by our assumptions. A classified list of the shades and tints of color which we have recorded for these F_2 hybrids is given in table 1.

One cannot ignore this series of colors on the assumption that the great variation in tones of color is due to external or ecological factors. We have grown F_2 plants in acid, in alkaline and in practically neutral soils; under varying degrees of moisture, light, and temperature; in the greenhouse and in the open. According to our records, only the colors listed have occurred under the various cultural conditions and, to date, no others. We found the color of corolla limb often very markedly influenced by the changing conditions; but if there was any change in pollen color we were unable to detect it. In flowers developed in absolute darkness, the pollen still has color but it is very much lighter in tone or shade. The evidence seems clear that the colors herein recorded are of genetic origin and point conclusively to a series of complex reactions within a group of color factors. To attempt to determine by selfings and crosses the exact genotype or genotypes of each shade or tint of color would be a time-consuming task that appears to us of doubtful value. We have, therefore, sought only such an explanation of the facts observed as will satisfactorily account for the ratio between the number of plants falling in each of the three main groups of spectrum colors and at the same time provide for an adequate variation in the hues of color which fall within the spectrum colors.

Records have been taken for 30 F_2 families comprising a total of but 684 plants. Of these, 102 plants were characterized by an entire absence of pollen. On the basis of the factorial analysis which we have suggested, the number of plants in each of the three color groups given in table 1 should be in the proportion of 48 blue to 160 green to 48 yellow. The ratio of the number of plants, and the number of tints, in the larger groups of spectrum colors as observed, compared with that computed, is given in table 2.

TABLE 1

*A complete list of colors occurring, in our cultures, in the pollen of the F₂ generation of P. axillaris × P. violacea, and the composition of each tint as determined by Ridgway.**

YELLOW			
	Percent		
Baryta Yellow	25 Orange+75 Yellow	O-YY	21 f
Pinard Yellow	25 Orange+75 Yellow	O-YY	21 d
Massicot Yellow	25 Orange+75 Yellow	O-YY	21' f
Naphthalene Yellow	100 Yellow		23' f
Barium Yellow	100 Yellow		23' d
Sulphur Yellow	75 Yellow+25 Green	YG-Y	25 f
Seafoam Yellow	75 Yellow+25 Green	YG-Y	25'' f
Pale Green-Yellow	55 Yellow+45 Green	G-Y	27 f
GREEN			
	Percent		
Pale Viridine Yellow	39 Yellow+61 Green	GG-Y	29 f
Glass Green	39 Yellow+61 Green	GG-Y	29'' d
Gnaphalium Green	39 Yellow+61 Green	GG-Y	29''' d
Pale Turtle Green	24 Yellow+76 Green	Y-G	31'' d
Light Turtle Green	24 Yellow+76 Green	Y-G	31'' d
Pale Fluorite Green	11 Yellow+89 Green	GY-G	33'' f
Light Fluorite Green	11 Yellow+89 Green	GY-G	33'' d
Clear Fluorite Green	11 Yellow+89 Green	GY-G	33'' b
Pale Olivine	100 Green		35'' f
Dull Opaline Green	93 Green+7 Blue	GB-G	37' f
Bluish Glauous	93 Green+7 Blue	GB-G	37''' f
Deep Bluish Glauous	93 Green+7 Blue	GB-G	37''' d
Pale Dull Glauous-Blue	75 Green+25 Blue	BB-G	41''' f
Greenish Glauous-Blue	75 Green+25 Blue	BB-G	41''' b
BLUE			
	Percent		
Pale Russian Blue	45 Green+55 Blue	BG-B	45''' f
Russian Blue	45 Green+55 Blue	BG-B	45''' d
Cadet Gray	45 Green+55 Blue	BG-B	45''' b
Pale King's Blue	25 Green+75 Blue	G-BB	47' f

* The symbols O-YY, YG-Y, etc. indicate the color relations in the color mixtures of each tint. The figures 21-47 indicate the steps in the spectrum scale.

The letters b, d, and f indicate the presence, respectively, of 9.5, 22.5 and 45 percent of white. The absence of a letter indicates full color.

The exponents ', ', and '' indicate the presence, respectively, of 32, 48, 77, 90 percent of neutral gray.

White weakens the spectrum color, gray dulls it.

It will be seen by reference to table 2 that there is in this instance a very close agreement between the actual observations and the calculations based on the assumption, as given earlier, of four allelic pairs of factors.

TABLE 2

Ratio of the number of plants in the 3 color groups, and the number of tints within each of these groups, in the pollen of the F_2 plants of *P. axillaris* × *P. violacea*.

Colors	RATIO OF NUMBER OF PLANTS				NUMBER OF TINTS		
	Blue	Green	Yellow		Blue	Green	Yellow
	101	: 379	: 102	Actual	4	— 14	— 8
	109	: 364	: 109	Calculated	4	— 17	— 8

Reciprocal crosses have been made between *P. violacea* and the F_1 plants of this series. The families in which the F_1 was used as the pollen parent have not yet flowered so that we can report at this time only on the cross of $F_1(P14 \times P9) \times P9$. Evidently it is immaterial so far as fruit and seed formation is concerned, whether *P. violacea* or the F_1 acts as the pollen parent. Ten families, comprising 209 plants, have been considered. Of these plants, 106 yielded no pollen. We have, therefore, but 103 records of pollen color for the 10 families observed. Theoretically the plants in this backcross should bear green and blue pollen in the ratio of 1:1. The actual and the computed results are given in table 3.

TABLE 3

Ratio of the number of plants in the 2 groups of color, and the number of tints within each of these groups, in the backcross of $F_1(P14 \times P9)$ to *P. violacea* and to *P. axillaris*.

Color	RATIO OF NUMBER OF PLANTS				NUMBER OF TINTS		
	Blue	Green	Yellow		Blue	Green	Yellow
$F_1 \times P9$	52	: 51		Actual	2	— 2	
	51.5	: 51.5		Calculated	2	— 2	
$P14 \times F_1$		189	: 453	Actual		6 — 7	
		321	: 321	Calculated		6 — 6	

Pollen color has been recorded for but one backcross to the F_1 in which P14 was used as the pollen parent. In this family but 7 plants matured. Because of the smallness of the family it was not included in this report. In general, we have not reported records of families yielding less than 10 plants, although the indications are that the ratios would not have been materially changed had the size of family been entirely ignored. The 7 families in which the F_1 was the pollen parent and *P. axillaris* the female parent gave rise to 642 mature plants and not one was characterized by abortive pollen. This backcross should give rise to plants bearing yellow and green pollen in the ratio of 1:1.

By reference to table 3, it is at once apparent that, in the backcrosses of the F_1 to *P. violacea*, both the actual ratios of plants and the number of

tints in the two color groups are in complete accord with the theoretical expectation. The unexpected excess of plants bearing yellow pollen in the backcrosses involving *P. axillaris* is interesting in connection with the unique type of sterility, referred to earlier, that is exhibited by *P. axillaris*. The anthers of P14 do not dehisce until after the corolla expands; but the pollen is apparently mature by the time that the flower bud has reached half the length that it attains before opening. One must, therefore, emasculate when the buds are comparatively small since later a slight pressure causes the anthers to burst, and some degree of selfing is almost sure to result. These backcrosses were made during the past three years, and, because of earlier experiences, the greatest of care was exercised in emasculating. We are, humanly speaking, certain that the great preponderance of yellow pollen in the progeny of this backcross has resulted neither from carelessness nor accident. The nature of the sterility displayed by *P. axillaris* together with the excess of yellow pollen recorded for this cross, suggests very strongly the presence in this species of selective fertilization. There may be some special physical or chemical attraction between the genes which unite at the time of fertilization in P14 to produce yellow pollen. So strong is this affinity that the pollen of *P. axillaris* does not function in fertilization in the absence of the possibility of the gene for yellow meeting another gene for yellow. In this case the eggs of P14 have equal chances to unite with sperms bearing the factor *B* or *Y*. It may be that the pollen tubes carrying the paternal cells which bear the factor *Y* are accelerated in their growth and penetrate the ovules in advance of the tubes with sperms carrying the factor *B*. This being so, a large majority of the seeds formed would carry the possibility of yellow pollen. On the other hand, it may be that there is a lack of affinity for, or that some form of repulsion exists between the gene for yellow and that for blue, or between the factor for yellow in P14 and certain combinations of factors occurring in the gametes of the F_1 .

In this backcross, 12 different tints of color are possible, 6 in the green and 6 in the yellow group. Although the proportion of plants bearing yellow pollen to those bearing green in this backcross was found to be far removed from expectation, the number of color tints listed in the cross, as shown in table 3, more nearly accords with the number obtained theoretically. Of the 7 tints of yellow and 6 tints of green actually recorded, as also in the 4 colors registered as occurring in the backcross to *P. violacea*, no color was observed which is not listed in table 1 as appearing in the F_2 generation. The presence of so large a number of colors in these backcrosses gives further evidence that more than a single pair of allelic factors is involved in the production of pollen color in the two species of *Petunia* under consideration.

The activities of the color factors are such that but 5 of the 29 colors which may occur in the second generation of this cross could be depended upon to breed true genetically; of the others, 9 tints would or would not breed true depending on the exact genotype selfed; and the remaining 13 colors could not be expected to breed true under any condition. A considerable number of F_3 families are being grown, but only 13 of them can be reported on at this time. Of these, 1 family, from an F_2 parent with Cadet Gray pollen, has bred true to the parental color; of 3 families from Naphthalene Yellow pollen, 2 have bred true and 1 produced pollen of various shades of yellow; 4 families, each from a different shade of yellow pollen, have produced only yellow pollen, but in varying shades; 3 families from different shades of green gave, each, pollen in shades of green and yellow; one family from Seafoam Yellow pollen has, to date, given only yellow and blue pollen; and a family from Pale Dull Glaucous-Blue pollen has given rise to blue and green pollen only. These results will be referred to again later. The study of the pollen of the F_3 generation of this cross must progress much farther before any adequate basis is secured for assigning the various tints of color in the F_2 plants to one or more specific genotypes.

PETUNIA AXILLARIS (Lam.) BSP.(P14) $\times$ P8

The pollen color of P8, the deep-purple cultivated strain, is Turtle Green. When this strain is crossed with P14, the pollen of the F_1 generation is invariably Light Turtle Green. Here again we have incomplete dominance, though a superficial observation might lead one to conclude that the pollen color is the same in the F_1 as in P8 since both are green. The F_1 pollen differs from that of P8, as based on Ridgway, only by the presence of a larger percentage of white; but this lighter green is very constant and characteristic. It may be considered as an intermediate color, but it has no additional yellow over that of P8, and none of the orange of P14 in its composition. The new color evidently results from a definite set of chemical reactions between the genes controlling pigment color in the pollen of *P. axillaris* and those genes responsible for pigmentation in the pollen of P8. In this cross, also, P14 of necessity served only as the female parent. But it was active as the pollen parent in the backcross to the F_1 generation.

The question at once arises as to the origin of the green pollen of P8. It is generally believed today that *P. axillaris* and *P. violacea* were lost to cultivation, having been superseded by their more attractive hybrids, some years before the close of the last century, and that they again attracted the attention of the gardener only in comparatively recent times. Strains of petunia breeding true for green pollen today may have arisen many years ago as the result of mutations in either one or the other of the original species, or in their cultivated progeny, at some time since these

species were first hybridized. On the other hand, they may be survivors of those early F_2 hybrids which normally bore green pollen.

If, following the assumptions given earlier, one determines the constitution of the 81 genotypes occurring in the F_2 generation of a cross between *P. axillaris* and *P. violacea*, it will be seen that certain individuals are possible in which the dominant factor for blue pollen does not appear. It is not only possible but very probable that our cultivated strain, breeding true for green pollen, had its origin in such a genotype. Among the many thousands of plants resulting from crosses between *P. axillaris* and P8 that we have grown, not a single F_1 , F_2 , F_3 , or F_4 plant has ever been found to bear blue pollen. We have, therefore, suggested that our large-flowered purple petunia (P8) is a direct descendant of an early hybrid of the genotype *bbDDGGyy*. But permanently removed from all influences on pigmentation exerted by the factor *B*, with which the factors *D* and *G* were originally associated in *P. violacea*, and under the varying conditions imposed by the gardener during years of cultivation, definite changes in the reaction of these color factors have taken place. Based on evidence from our cultures, it becomes apparent that certain mutations have occurred which affect both the actions and the interactions of the genes involved.

D, *G* and *Y*, as in the species cross, continue to act as incomplete dominants. However, *G* has assumed a more active rôle in pigmentation and is no longer hypostatic to *Y*; but homozygous *Y* is now revealed as a stronger pigment factor than homozygous *G*, so that *GGYY* produces yellow pollen with a green tone. On the other hand, due to the slight degree of incomplete dominance of *G* as compared with that of *Y*, *GG* or *Gg* with *Yy* produces yellowish green tints. The homozygous recessives, *ggyy* like the homozygous dominants, give a greenish yellow, which, in the case of the recessives, is a very light tint.

The change in *D* has been very slight and only such as to emphasize, somewhat, its activity in pigmentation. When homozygous, and in the absence of all other dominants, *D*, in its slightly modified rôle, gives rise to gray pollen.

The recessives remain unchanged in their influence on pollen color with the exception of *b* which has been practically reduced to white and serves only as a weak dilution factor.

The number of chromosomes in P8 is the same as in the species and there are no morphological differences, which can be detected by use of the microscope, between the individual chromosomes of *P. axillaris*, or those of *P. violacea*, and the chromosomes of the cultivated strain. The modifications which have taken place in the genes *b*, *D*, and *G*, as indicated above, are apparently not due to chromosome changes. They are rather to be considered as the results of gene mutations, the mutations having to do chiefly with the intensity of the reactions of the genes affected.

When P14 with its Pinard Yellow pollen (*bddggYY*) is crossed with P8 with its Turtle Green pollen (*bbDDGGyy*), the F_1 pollen is Light Turtle Green in color and is represented by the genotype *bbDdGgYy*. The pollen of the F_2 plants shows as great a range of variability in color as that exhibited by the pollen of the second generation of the species cross. In this case, as noted earlier, there is an entire absence of blue and the various shades and tints of color fall into the three groups—green, yellow, gray. The various tints recorded as occurring within each of the main color groups in the F_2 plants of this cross and the composition of each tint, as based on Ridgway, is given in table 4.

TABLE 4

A classified list of the colors observed in the pollen of the F_2 generation of P14×P8, and the composition of each shade and tint as determined by Ridgway.

YELLOW				
Percent				
Baryta Yellow	25 Orange+75 Yellow	O-YY	21	f
Pinard Yellow	25 Orange+75 Yellow	O-YY	21	d
Massicot Yellow	25 Orange+75 Yellow	O-YY	21'	f
Martius Yellow	100 Yellow		23	f
Naphthalene Yellow	100 Yellow		23'	f
Barium Yellow	100 Yellow		23'	d
Sulphur Yellow	75 Yellow+25 Green	YG-Y	25	f
Pale Greenish Yellow	75 Yellow+25 Green	YG-Y	25	d
Light Greenish Yellow	75 Yellow+25 Green	YG-Y	25	b
Pale Chalcedony Yellow	75 Yellow+25 Green	YG-Y	25'	f
Seafoam Yellow	75 Yellow+25 Green	YG-Y	25''	f
Pale Green-Yellow	55 Yellow+45 Green	G-Y	27	f
GREEN				
Percent				
Pale Viridine Yellow	39 Yellow+61 Green	GG-Y	29	f
Light Yellow Green	24 Yellow+76 Green	Y-G	31	d
Pale Turtle Green	24 Yellow+76 Green	Y-G	31''	f
Light Turtle Green	24 Yellow+76 Green	Y-G	31''	d
Turtle Green	24 Yellow+76 Green	Y-G	31''	b
Light Fluorite Green	11 Yellow+89 Green	GY-G	33''	d
Clear Fluorite Green	11 Yellow+89 Green	GY-G	33''	b
Pale Olivine	100 Green		35''	f
Dull Opaline Green	93 Green+ 7 Blue	GB-G	37'	f
Dark Bluish Glauous	93 Green+ 7 Blue	GB-G	37'''	b
Greenish Glauous-Blue	75 Green+25 Blue	BB-G	41'''	b
Bluish Gray-Green	75 Green+25 Blue	BB-G	41'''	
GRAY				
Percent				
Gull Gray	58.5 Black+41.5 White	Carbon Gray		d
Deep Gull Gray	67.5 Black+32.5 White	Carbon Gray		b

Records have been taken for 60 F_2 families of this cross totaling 7153 plants. Of these, 673 bore no pollen while 6480 produced pollen in abundance. On the basis of our assumptions the number of plants in each of the three groups of colors should be in the ratio of 108 yellow to 144 green to 4 gray. These F_2 plants should give rise to 27 shades and tints of color. The ratio of the number of plants, and the number of tints, occurring in each of the three groups of color, as compared with that expected is given in table 5.

TABLE 5

Ratio of the number of plants in the 3 groups of color, and the number of tints within each of these groups in the pollen of F_2 ($P_{14} \times P_8$) plants.

Color	RATIO OF NUMBER OF PLANTS				NUMBER OF TINTS		
	Gray	Green	Yellow		Gray	Green	Yellow
	99	: 3472	: 2909	Actual	2	— 12	— 12
	101	: 3645	: 2734	Calculated	1	— 12	— 14

The results, as given in table 5, show a rather broad discrepancy between the actual and the expected ratios. But this is not surprising when one bears in mind that they include the first plants for which records of pollen color were taken. A considerable proportion of the records for this cross was, therefore, made before the more refined methods, outlined earlier, for matching pollen to the Ridgway colors, had been adopted. Undoubtedly there was a failure in the early observations to distinguish clearly between certain greens and yellows.

In 2 of the backcrosses made between P_8 and $F_1(P_{14} \times P_8)$ the F_1 served as the pollen parent and in 6 other cases the pollen was furnished by P_8 . The 8 families resulting from these pollinations comprised 973 plants of which but 777 were pollen-bearing. These plants should produce green pollen only and in 8 different shades. It will be seen by consulting table 6 that the theoretical expectations correspond perfectly in this cross with the results obtained. Our first backcross in this series was made in 1926. In this instance, P_8 was used as the pollen parent. An exceptionally large family resulted. Of the 442 plants that produced pollen, 210 bore various shades of yellow pollen and 232 produced green pollen in varying shades. These results are more nearly what would be expected in an F_2 family and are manifestly due to careless manipulation in pollination. For this reason, together with the fact that the other 8 families gave only green pollen, the data for this largest family were not included in the summary of this backcross as given above and in table 6.

In the backcrosses to *P. axillaris*, the $F_1(P_{14} \times P_8)$ served as the pollen parent for 7 families and P_{14} for 1 family. These 8 families produced 3008 pollen-bearing plants which were divided into 2 groups as regards pollen

color. These plants should bear yellow and green pollen in the ratio of 3 to 1 and there should be 2 shades of green and 6 shades of yellow. The actual and the expected results in this cross are given in table 6.

TABLE 6

Ratio of the number of plants in the color groups, and the number of tints within each of these groups, in the backcross of P14 and P8 to F₁ (P14×P8).

Color	RATIO OF NUMBER OF PLANTS			NUMBER OF TINTS	
	Yellow	Green		Yellow	Green
P14×F ₁	2271	: 737	Actual	6	— 2
	2256	: 752	Calculated	6	— 2
P8×F ₁	0	: 777	Actual	0	— 8
	0	: 777	Calculated	0	— 8

The very close parallel between the results actually obtained in our cultures for the backcrosses of this series of hybrids, as shown in table 6, and the theoretical expectations further substantiate our assumption that at least four pairs of allelic factors are involved in the inheritance of pollen color in the two species *P. axillaris* and *P. violacea*; and that the cultivated strain with green pollen may represent an early segregate from a cross between these two species.

Following out the factorial analysis herein presented, in an attempt to explain the method of inheritance of pollen color in *Petunia*, it becomes clear that in some cases a given color may be represented by several genotypes, and thus, as stated earlier, may or may not breed true. To test this point, each plant of an entire F₂ family was selfed, resulting in over 8000 plants. The results show very conclusively that a given color of pollen may be represented by two or more genotypes. To illustrate, of 27 families secured from plants bearing Turtle Green pollen, 5 bred true to Turtle Green, 2 gave only green but in varying shades, 3 produced shades of yellow and green, and 17 produced, each, plants with a full range of yellow, green, and gray pollen. These 27 families were all secured from different plants of the same F₂ family. Only 2 plants of this F₂ family bore Greenish Glauous-Blue pollen. These were selfed and both F₃ families came true to the parental color for pollen. It will be recalled that the pollen of the F₁ plants resulting from crossing P14 and P9 is Greenish Glauous-Blue. All the factors for pollen color must, in this particular case, be heterozygous and the plants could not breed true for pollen color. These results indicate that one would need to repeat tests many times, in seeking to determine the constitution of the various phenotypes for pollen color, before one could be sure that the particular phenotype under consideration was repre-

sented by a single genotype, homozygous for all its factors, and by no other genotypes. Additional evidence of this same fact was given earlier in regard to the F_3 plants of $P14 \times P9$.

The record of observations as outlined and discussed in this paper would seem to justify the conclusion that at least 4 allelic pairs of factors are operative in the production and inheritance of pollen color in the F_1 plants, resulting from crossing *Petunia axillaris* and *Petunia violacea*, and in certain of their progeny. At the same time it offers a reasonable explanation of the origin and color behavior of green pollen in a cultivated strain of *Petunia*, and demonstrates the possibility of a single shade or tint of pollen color being represented by two or more genotypes.

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