COLORATION OF THE SEED COAT OF COWPEAS

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INTRODUCTION

The following study of the seed coat of various cultivated cowpeas (Vigna sinensis) was made because the great diversity in color schemes and kinds of pigment in these seeds seems to have a direct bearing on problems of heredity, the pigmentation being to a large extent a basis for distinguishing one variety from another. It therefore seemed desirable that a clear understanding of the morphology of the seed coat and the way in which these pigments are arranged in its various layers should be obtained, in order to discover whether there are any facts bearing on problems of heredity, outside of the mere facts of the different color arrangements themselves. It also seemed not unlikely that such a study might prove that colors optically alike are in some cases different as to the material of the pigment and the place of its deposit.

METHODS OF PREPARATION

The best methods for the study of the cowpea pigmentation were found to be as follows:

The seed coats were removed from dry cowpeas in flakes as large as possible and were then cut transversely in pith, the sections made by hand being as thin as possible. It was found that to embed the seed coats for microtome sectioning necessitated subjecting them to water if the freezing process was used or to various solvents if celloidin or paraffin was used. Both methods resulted in dissolving the pigments to some extent and thereby causing them to appear in parts of the seed coat where they did not normally belong. The dry method of cutting avoided this difficulty. The sections were mounted dry under ½-inch square cover glasses, held in place by a drop of paraffin on either side of the glass. By this method the sections may be examined in the dry state and closely watched when water, various reagents, or stains are being applied, so that facts as to solubility, chemical reaction, etc., may be accurately noted. Such sections, held down by an immovable cover glass, are also ideal for high-power examination. If necessary, they may be also readily preserved for future study. Sections tangential to the surface of the seed coat were also made, but, aside from throwing light
upon the structure of the cells in the various tissues, it was found that they were not so useful for an examination of the pigments and their distribution as sections made transverse to the seed coat or, in other words, perpendicular to the surface of the cowpea.

A large number of reagents were experimented with, but eventually it was found that those of practical use were extremely few—namely, distilled water, alcohol, ether, chloroform, xylol, solutions of caustic soda and caustic potash (the 1 per cent solutions being of greatest service), dilute hydrochloric acid, normal Fehling's solution, saturated aqueous solution of chloral hydrate, peroxid of hydrogen solution, and several stains, the most satisfactory being a 50 per cent alcohol solution of diamond fuchsin and a 10 per cent aqueous solution of pyronin. As above stated, these were used upon sections mounted dry under the cover glass, the various liquids being drawn through by means of triangular pieces of blotting paper placed at the opposite side of the cover glass. The length of treatment varied under different circumstances from a few seconds to 24 hours. However, most of the reactions that were significant were obtained within a few minutes, so that study could be rapidly carried on.

As the problems in mind had to do with the differences in the color schemes of cowpeas as a whole, only a general examination was made to learn in what respect different areas of the seed coat were differently pigmented. It became evident that although the pigment intensification varied in different areas of the seed coat, a general idea of the color scheme could best be found by studying sections taken from the side of the seed. The greater intensity of color around the hilum was found to be merely due to a larger quantity of the same pigments as those present on the side of the seed, and very frequently this heavier pigmentation proved to be a disadvantage, as, in the case of dark colors, they frequently obscured less intense pigments easily detected in sections made where coloration was not so dense. The only case where the pigmentation near the hilum was particularly worth studying was in those varieties where colors on the general surface were lacking—namely, in the cream-white and pure-white varieties. In this class the varieties that have a more or less intense pigmentation around the hilum give some information as to the tendency of general pigmentation that such a variety might be expected to show were the whole of the seed coat colored in the usual way.

MORPHOLOGY OF THE SEED COAT

Before discussing the coloration schemes in different varieties of the cowpea it is necessary to describe the structure of the seed coat, especially as seen in transverse sections. Such sections show that it may be divided into three layers. The outer layer is a single row of elongated palisade cells, with their long axis perpendicular to the surface of the seed
(fig. 1, a). Beneath this is a second layer, a single cell deep. Its cells are relatively cubical and have thick walls, but one horizontal diameter is slightly longer than the other. They are described by some authors as of hour-glass shape, a rather fanciful resemblance. They may be said to lie at right angles to those of the palisade layer (fig. 1, b). Beneath this second layer is a comparatively thick layer from 10 to 20 cells deep, the cells being larger than those of the second layer and with relatively thin walls. These also lie parallel to the surface—that is, are hori-
horizontal. They are considerably longer than broad and are, as a rule, so arranged that their longer horizontal axis is at right angles to the slightly longer horizontal axis of the cells of the second layer (fig. 1, c). One of the advantages of this arrangement may be to give tensile strength to the seed coat.

The middle layer of relatively cubical cells plays no rôle whatever in the pigmentation of the cowpea. Its cells are practically empty, only such residue of organic matter being present as would necessarily be found there. It is possible that there is some effect upon the coloration due to the included air which fills these cells and which in fresh-mounted sections always appears like a black band through the section; but, as the resulting color of the seed coat is made up from the different factors taken vertically, this single-celled empty layer between the palisade and the basal layers must have extremely little influence upon the color.

**PIGMENTATION OF THE BASAL-COLOR LAYER**

The third or inner layer is more or less filled with a pigment, which is the same in all the cowpeas examined, and for that reason the writer calls this the basal-color layer. The pigment is a melanin-like substance, ranging from a pale-straw color to a deep orange or heavy buff. As a rule, it is massed in granular particles in the lower part of the layer. In some cases the upper cells contain the larger amount of pigment, and in a few instances it is evenly distributed throughout all the cells of the basal layer. In some cowpeas, and especially in those that have a heavy basal color, the pigment completely fills a large part of the cells and is then seen to be crystalline. In such cases the color is a deep orange or sometimes even a copper red. No trace in any instance was found of any other pigment in this basal layer. Anthocyanin tests failed in every case to give a reaction.

No attempt is made in this paper to discuss the cell contents of the seed coat, outside of those substances which are directly concerned in producing the color schemes found to exist in ripe cowpeas. How the various pigments arise in the growing cells and what are the mechanical principles back of their predetermined distribution in the different varieties are questions of great cytological interest, but not important for the subject in hand. It may, however, be worth while to mention here one substance which is very generally associated with the different pigments—namely, tannin. Tests with such reagents as ferric chloride, ferric acetate, potassium bromate, osmic acid followed by hydrogen peroxide are usually successful in bringing out the presence of tannin in cowpea seed coats.

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1 The applying of the term "melanin" to any plant pigment has been criticized. (See Gortner, R. A. The misuse of the term "melanin." Science, n.s., v. 36, no. 915, p. 52-53. 1912.) Although Mr. Gortner, so far as the writer knows, has not sufficient ground to warrant his exclusion of this term from plant nomenclature, seeing that the statement that it never occurs in plants is unproved, the writer agrees that its use here is open to criticism, and has therefore substituted "a melanin-like pigment" because no advantage can be found in employing Osborn's term "humin," favored by Gortner, the boundaries of this term being at present as vague and unsettled as those of melanin.
peroxid, or any of the alkaline carbonates demonstrate that one or more of the half-dozen tannins known to exist in plants are present in appreciable quantity in all the pigmented cells of the cowpea. But the optical color effect of this tannin is too small to need any attention here. Whatever rôle tannins may play in producing the pigments deposited in the cells, their chief service in the mature seed coat is undoubtedly protective.

PIGMENTATION OF THE PALISADE LAYER

The upper or palisade layer plays a most important part in the pigmentation of the seed coat. As a rule, the cells are from 6 to 10 times as long as broad, with the cell cavity greatly enlarged at the lower or inner end, and gradually tapering upward to a mere thread at the upper or outer end of the cell (Pl. VI, fig. 1, a, and text fig. 1, a). From the central cavity, however, there radiate out into the gradually thickening walls from three to six vertical clefts, each reaching or nearly reaching the cell wall, thereby affording intercommunication between the cells. The result of this is that, looking on the cowpea seed coat from above, these cells appear to have at their outer end a stellate cavity (fig. 2). In focusing downward on the palisade cells these clefts gradually decrease by the widening of the central cell cavity until at or near the base the

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1 See an article on anthocyanin-forming bodies by Ioannes Politis, entitled "Sopra speciali corpi cellulari che formano Antocianine," published in Atti, R. Accad. Lincei, s. 5, Rend., Cl. Sci. Fis., Mat., e Nat., v. 20, sem. 1, p. 838-842, 1911. Politis here claims to have proved that anthocyanin is produced by certain special organs, cyanoplastids, in the composition of which tannin is a chief ingredient.
cavity occupies almost the entire width of the cell. The walls are of exceedingly dense cellulose. An outside cuticular sheath in rare cases is found covering the upper or outer surface of the palisade layer, but in most instances it is lacking (Pl. VI, fig. 1, cu), and the narrowed cavity of the cell seems to either reach or almost reach the outer surface of the seed coat. That there often is an actual aperture at the upper end of the cell is easily demonstrated; for when stains or colored reagents are used, it is easy to trace the rapid inflow of the liquid through these narrow, threadlike extensions of the cell cavity downward into the larger area at the lower end. Air bubbles are also seen to be pushed forward by the inflowing liquid and to pass downward into the large basal cavity. The bearing of this fact on the absorption of moisture necessary for the germinating of the seed is evident.

The normal form of the palisade cells in some varieties is strangely modified, the cell walls being very irregular. The taper of the cell cavity in such cases is imperfect, and after suddenly narrowing from the wide basal portion to a mere thread it again expands toward the outer end into a sort of mushroom-shaped enlargement. The cells themselves are also greatly contorted in outline and are sometimes spirally twisted upon their long axis, so that a true longitudinal section of these cells, such as is usually obtained in a transverse section of the seed coat, is quite impossible. Sometimes they are more or less intertwined. These distortions, as will be pointed out, are associated with certain color schemes and are quite characteristic of certain varieties.

There are two classes of pigment found in the palisade cells: First, a melanin-like pigment, identical in all its reactions and similar in its color to the pigment referred to in the lower of the three layers—namely, the basal-color layer. In some instances this is present in all the palisade cells, thereby supplementing and intensifying the basal color of the seed coat. In most cases it is confined to small groups of cells interspersed among larger or smaller areas of the palisade layer destitute of this pigment. According as this pigmentation is uniform or irregular in its deposit the basal color of the seed coat is uniform or mottled.

More frequent than this melanin-like pigment in the palisade layer are various anthocyanin pigments. These also may fill uniformly all the palisade cells or may be variously grouped and interspersed with colorless cells, thereby giving rise to the very diverse color schemes characteristic of different varieties of cowpeas. The anthocyanin pigments are practically of two kinds: First, an acid-reacting anthocyanin, ranging in color from a decided rose red to a strong purple; and, second, an alkaline-reacting anthocyanin, uniformly of a deep indigo blue, but which in mass often appears as dead black.

In many instances only one of these phases of anthocyanin pigment is discoverable in the seed coat of a given variety; and according as it fills
uniformly the cells of the palisade layer or is irregularly deposited, we have modifications in the color of the seed coat, giving rise to various forms of speckling, blotching, marbling, or monochrome coloration. In many cowpeas both the alkaline-reacting and the acid-reacting anthocyanin are present. As a rule, they are deposited in separate cells, but in a great many cases they are to be found in the same cell. When this latter is the case, the alkaline-reacting anthocyanin always occupies the lower half or third of the cell—that is, the part where the cavity is largest—and is collected in dense granular masses of a deep indigo blue. The rose-colored anthocyanin usually occupies the upper portion of the cell or occasionally fills more or less the entire cavity. The finding of both alkaline and acid reacting anthocyanin in the same cell is in harmony with a well-established cytological condition, namely, that one end of a cell may give an acid reaction while the other gives an alkaline one.

That these two phases of anthocyanin pigment are probably the same material is easily demonstrated. When thin transverse sections are infused with neutral distilled water, the rose or purplish anthocyanin generally found in the upper end of the cell quickly diffuses into the surrounding liquid, thereby rendering more visible any alkaline indigo-blue anthocyanin which may occupy the lower portion of the cell. This latter, although also soluble in water, is very much slower in dissolving, taking several hours to disappear. Moreover, if such sections, instead of being treated with distilled water, are treated with a weak alkaline solution, such as a 1 per cent solution of caustic potash, both phases of anthocyanin undergo the same reactions. The rose-colored anthocyanin is immediately changed into an intense blue, and this, together with the indigo-blue anthocyanin, slowly passes through different shades of blue, green, greenish yellow, pale yellow, and finally is bleached and disappears. If, on the other hand, a weak solution of an acid is used, such as a 1 per cent solution of hydrochloric acid, both phases of anthocyanin again undergo the same reaction. The indigo blue immediately changes to an intense rose red and rapidly diffuses in the surrounding liquid. The tints assumed by these two phases of anthocyanin pigment are so perfectly identical with all the reagents that have been tried that it is fair to assume that we have essentially the same material in both cases, but in the one instance in an acid state and in the other in an alkaline state.

It will be seen that in all pigmentations of the palisade layer the colors are superimposed upon the underlying basal color of melanin-like pigment. It is by means of this palisade layer, therefore, that we secure the great diversity in color schemes characteristic of the cowpea. If the general pale-buff or orange-brown basal color is modified by even deposits of melanin-like pigment in the palisade layer, an intensification of the basal color is obtained, which sometimes amounts to a copper
red or dull reddish brown, uniformly spread over the seed coat. If the palisade layer contains uniformly anthocyanin in its cells, the basal color is obscured or modified by this superimposed pigment and assumes a blue or black or purple tint. If the palisade cells are irregularly pigmented, all of the modifications in marbling, speckling, and streaking which serve to distinguish the different varieties of cultivated cowpeas are found (Pl. VI, fig. 2). It may therefore be said in general that the diversification in color is principally brought about by the deposit in the palisade cells alone of the pigments above mentioned and that the different tints of color tones are the result of the various combinations of pigment already mentioned.

SEEDS DESTITUTE OF PIGMENTATION

Some reference should be made to such cowpeas as are more or less destitute of pigmentation. The writer examined only one cowpea the entire seed coat of which gives no evidence of pigment deposit; but among the large number of white and cream-white cowpeas there are probably several others quite destitute of pigmentation—that is, true albinos. In fact, four other varieties of white or cream-white cowpeas were examined in which the maximum quantity of pigment was so minute as to make its detection quite difficult, and certain individuals of these varieties, after the most painstaking tests, left the question in doubt as to whether or not even a trace of pigment was present. They were Nos. 212–2–11, 212–6–8, 213–2–4, 214–3–2Re. From the standpoint of coloration, therefore, these pure white and cream-white varieties may all be safely considered as albinos. The strict albino examined was No. 0632. Most careful testing failed to disclose any coloration in the palisade layer or in the basal-color layer, long treatment with various reagents resulting merely in such faint tints as would be secured by reactions on the normal cell contents, such as cytoplasm and nuclear substances. With the exception of these albinos, all the cowpeas examined have more or less pigment deposited in the basal-color layer, and, as before stated, this is of a melanin-like character. When the palisade layer is destitute of pigment, uniform tinted cowpeas are obtained, ranging from a cream-white seed coat, where the amount of pigment in the basal-color layer is very small, to a strong buff or even red brown, where the amount is greater.

After this general consideration of the structure of the seed coat and of the various ways in which its color layers are pigmented, the chief varieties of cultivated cowpeas may be described individually. The varieties here enumerated represent in a general way all the known color schemes found in cowpeas. They were chosen at the suggestion of Prof. W. J. Spillman, of the Bureau of Plant Industry, who proposed this line of investigation, these types being those used by him in connection with certain studies in hybridization.
CLASSIFICATION OF COLOR FACTORS IN COWPEAS

For convenience of reference, the foregoing factors of coloration in cowpeas may be classified as follows:

I. Basal-color layer.
   a. Devoid of pigment (white).
   b. With melanin-like pigment (buff to brown).

II. Palisade layer.
   A. Solid colors.
      a. Melanin-like pigments.
         a'. Buff, clay, etc.
         b'. Coffee, brown, etc.
      b. Anthocyanin pigments.
         a'. Red (acid state).
         b'. Blue (alkaline state).
         c'. Purple (combination of a' and b').
         d'. Black (intensification of b' or c').
   B. Variegated colors.
      a. Marbling.
         a'. Affects melanin-like pigments only. Whippoorwill type.
      b. Speckling.
         a'. Deep-blue anthocyanin in groups of cells; groups widely scattered.
            Taylor type.
         b'. Same, but groups more plentiful. New Era type.
      c. Marbling and speckling.
   C. Eyed.
      a. Watson type of eye. Margin of eye indefinite.
      b. Holstein type of eye. Seed of eye indefinite.
      c. Narrow eye. Narrow patch of color about the hilum indefinite at lower end (micropylar).
      d. Small eye, due to presence of both a and b.
      e. Very small eye, due to the presence of both a and c.
   D. Dilute colors.
      Characterized by individual unpigmented cells scattered among pigmented cells over entire seed coat.

CLASSIFICATION BASED ON DISTRIBUTION AND KINDS OF PIGMENTS

For convenience in grouping, the writer has divided the different varieties into four classes: (1) Those with or without a pigment in the basal-color layer, but none in the palisade layer; (2) those in which there is a pigment in the basal-color layer and anthocyanin only in the palisade layer; (3) those in which there is pigment in the basal-color layer and a melanin-like pigment only in the palisade layer; and (4) those in which there is a pigment in the basal-color layer and both anthocyanin and a melanin-like pigment in the palisade layer.

1.—COWPEAS HAVING NO PIGMENT IN THE PALISADE LAYER

Under the first division I have found, as previously stated, one cowpea, No. 0632, which is an extreme type of albinism. Here both the palisade and basal-color layers are destitute of all pigmentation. The
writer has not been able to discover the parentage of this variety. The reasons for its white color are two: First, the palisade cells are practically destitute of contents. Such residuary amount of cytoplasm as is present occupies a very minute part in the cell cavity and generally its upper third, instead of being in the lower end, as is usually the case. Parallel with this fact the usual spindle-shaped tapering of the cell cavity is here so slight that the diameter of the lower part is hardly greater than that of the upper part. In other words, the fine hairlike extension of the cell cavity upward does not exist. Near the upper part of the cell the somewhat narrow canal cavity widens out, and it is at this point that the small residue of cytoplasm is to be found. The cells are also more loosely bound together than usual, so that intercellular spaces between them are quite frequent. The second reason for the white color is that in the basal-color layer, which in most cowpeas is colored with a dense yellow or orange-buff pigment, there is no trace of pigment present nor any pigment reaction obtainable. The seed coat is, as a whole, much thinner and weaker than in other varieties, and its permeability to external moisture should therefore be greater.

As already stated, several other cowpeas approximate this true albino in being practically colorless, but certain individuals of these varieties show a slight trace of pigmentation in the basal-color layer. These varieties, 212–2–11, 212–6–8, 213–2–4, and 214–3–2Re, have the striking irregularity in form of the palisade cells and the lack of taper in the cell cavity just described in the case of No. 0632. Special mention should also be made of a somewhat analogous case in No. 239–5–3–18. This is also a cream-colored cowpea, but has a deep purplish pigmentation around the hilum, forming an "eye." In view of this localized pigmentation it is necessary to classify this variety under Division IV, the palisade cells in the area of the "eye" having both anthocyanin and melanin-like pigment. The color scheme of this portion of the seed coat will therefore be treated under Division IV, but as the structure of the uncolored seed coat, exclusive of the "eye," shows certain curious features identical with the white forms just described the case is here given for comparison. A transverse section of the cream-white seed coat of this cowpea shows remarkable contortion in all its layers. The palisade cells have very strongly marked the abnormal shape previously mentioned, having heavy walls and being shorter than usual; their form is irregular and twisted upon its axis. The cell cavity is very broad at the base, narrows suddenly at its middle, and again broadens slightly at the upper end. The very small residue of cytoplasm is generally found located in this upper widened portion, thus corresponding to the albino, No. 0632. Here also there is no appreciable trace of pigment to be found in the palisade cells. The cells of the remaining layers of the seed coat are also much contorted and have, in general, heavier walls than normally. A minute amount of pigment is present in the
basal-color layer and is contained in widely separated cells. It gives the same reaction as the yellow melanin-like pigment usually found in this layer. It is here, however, of a very light-straw color, this being due to the minute quantity rather than to any difference in character. This hybrid is the third generation of a cross between a Watson No. 5 and a Taylor No. 14. The significance of the contorted cells here mentioned should be borne in mind in view of its parentage, as it will be a subject for discussion under a later variety.

No. 237–3–7 is in its general color cream white, often intensified into buff, or even in a few individuals distinctly brown. The color is more conspicuous about the hilum. Therefore, it should be classified and described under Division III, although in general appearance it often seems to be uncolored.

II. COWPEAS HAVING ONLY ANTHOCYANIN IN THE PALISADE LAYER

The second group of cowpeas is that having only anthocyanin in the palisade cells, with a melanin-like pigment always present in the basal-color layer. Nine varieties were found to have enough difference in color scheme to be separately examined. In the first, No. 243–1–5, the seed coat is a strong red, varying to purplish brown. The palisade cells are strongly pigmented with the general color of the seed coat, so that the basal-color layer, which has the usual orange-yellow pigment, probably has little part in the general coloration, being obscured by the heavy pigmentation of the palisade layer overlying it. In neutral water the palisade pigment appears as a dull rose and slowly dissolves. Various reagents show it to be anthocyanin. Possibly it is mixed with a minute trace of buff-tinted melanin-like pigment, for there seems to remain a faint suggestion of a dull-buff pigmentation in the palisade cells after the anthocyanin has been removed.

The basal-color layer is a strong orange yellow, the pigment being the melanin-like material usually found in this layer. This variety is the second generation of a cross of Red No. 4 on Whippoorwill No. 6.

No. 253–2–3B–23 is a cowpea having a general blue-black tint, due to a speckling of deep blue on a ground color of light or dark brown, the latter being more or less obscured by the darker color. In sections treated with neutral water this pigment, an anthocyanin, shows as a strong indigo blue, confined principally to the lower ends of the palisade cells. No trace of rose-red coloration was found. Decolorized sections, if stained with diamond fuchsin, show an intensity of stain in proportion to the degree in which the cells were pigmented, and it is then more clearly seen that a fair proportion of these cells, certainly more than one-half, are without this pigment. No indication of any melanin-like pigment is found in the palisade layer. With hydrogen peroxid and ferric sulphate the palisade layer is rapidly bleached, but the basal-color layer resists the
action of this powerful liquid for some time. This layer in water shows as a deep orange-buff color, due to the melanin-like pigment generally found in this layer.

The scheme of coloration is produced by the blue-black pigment above mentioned superimposed upon the strong-clay or light-coffee color found in the basal-color layer. The strong pigmentation around the hilum is identical in character with that found on the rest of the seed coat. This hybrid is the second generation of a cross between a blue Taylor No. 20 and a Red-Eye No. 26.

A very similar cowpea in general color scheme is the so-called blue Macassar No. 21299a. The seed coat ranges in various individual cowpeas from an almost purple blue to a complete black. Transverse sections of the seed coat show that the pigment, a blue black, is somewhat unevenly distributed throughout the palisade cells, although no cells seem to be absolutely destitute of it. Associated with this blue black is a small quantity of rose-colored pigment which occupies the upper part of the cell, the deep blue being uniformly found in the lower one-third. This rose-red pigment, an acid anthocyanin, is quite evenly distributed through the palisade layer, so that the inequality in the pigmentation of the seed coat, which may be detected with a hand lens, is due to the unequally deposited deep-blue, alkaline anthocyanin.

A mere suggestion of a faint buff pigment was detected in the palisade cells, presumably the melanin-like material found in this position in other cowpeas. But as the quantity, if present, is too small to play any rôle in the coloration and its presence in any quantity is unverified, it may be left out of consideration. It should also be stated that in this cowpea a test for tannin shows that an unusual quantity of some one of this group of compounds is present in the palisade cells.

The basal-color layer is strongly pigmented with the usual deep yellow melanin-like substance. The intense color superimposed upon this by the contents of the palisade layer probably prevents its having any considerable effect in the exterior coloration of the seed coat.

This variety was secured from Piracicaba, Brazil, May, 1907. There are no data on its parentage. It may be stated that in Brazil all varieties are called "macassar."

In No. 227-2-4, a black cowpea, all of the cells of the palisade layer are well filled, at least in the upper two-thirds, with a rose-red acid anthocyanin. Somewhat less than one-half contain also an extremely dense and granular indigo-blue alkaline anthocyanin. In cells destitute of the latter the rose anthocyanin fills the entire cell. Were it not for the intense coloration due to the rose anthocyanin the unequal distribution of the indigo-blue pigment would result in a blotched or speckled condition of the seed coat. The basal-color layer is a pale-straw tint or sometimes merely a cream white. The palisade cells are free from all con-
tortion or other modification of form. This hybrid is the second generation of a cross of Watson No. 5 on Coffee No. 27.

No. 228-5-4 is usually black, but shows great variability in its color scheme, ranging from uniform black into black with small irregular fawn-colored or reddish brown marbling, or a fawn and reddish brown marbled with black, or having blue-black speckles, or in rare instances the entire cowpea is a uniform fawn or light red brown, especially immature seeds. There are, therefore, three pigment elements to be considered: (1) Deep blue or black areas, (2) blue-black speckled areas, and (3) fawn to red-brown basal areas.

The deep-black areas show that this color is an intensification of a strong purple in the pigment cells, which, although it is present in all the cells of such areas, is still quite variable as to quantity, some cells being pigmented only in the extreme lower end and others through the entire cell cavity. On treatment with neutral water this color resolves into the two factors before noticed—namely, a rose-colored anthocyanin very uniformly distributed throughout this layer and an indigo-blue anthocyanin massed in the lower end of certain cells. It is therefore evident that these black areas would show a somewhat mottled condition if the excessive pigmentation did not obscure this.

The second pigment element—namely, the blue-black speckling—shows a very different condition of things. This also is due to an anthocyanin deposit in the palisade cells, but it is clearly distinguishable from what was found in the solid black areas in four respects: (1) The color is always a vivid indigo blue, not a purplish blue, nor does it give a rosy diffusion in water; (2) the quantity of this blue pigment is very much greater in the cells producing the speckling than in those found in the solid black areas; (3) it always extends upward toward the top of these cells, instead of being segregated into a heavy mass at their base; (4) the cells containing this particular pigment are in small groups, not solid masses as in the black areas, but usually large enough to be seen by the unaided eye. This is the Taylor or New Era type of speckling.

The third and remaining color is that of the fawn or reddish brown basal tint, which is due to the usual melanin-like compound contained in the cells of the basal-color layer. It ranges from a faint yellow to an intense yellow or even a copper color. This variation in quantity is the cause of the difference in color of individuals, ranging from pale fawn to reddish brown.

The palisade cells are strikingly regular, straight in outline, narrow in diameter, and long. The entire seed coat is somewhat heavier than usual. The grandparents are Clay No. 17 crossed on Coffee No. 27.

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1 Prof. W. J. Spillman has informed the writer that among cultivated cowpeas these black areas occur only in hybrids having all the factors for black pigment and having also the factor for the Taylor or the New Era style of speckling, and also that such a type can not be fixed in cultivated cowpeas, although it is the normal condition in wild cowpeas.
No. 239–4–3–6 is what is known as a Holstein pattern, a cream-white basal color blotched with large masses of black. Transverse sections through the black areas show that the pigmentation scheme is similar to that already seen in the black areas of varieties above mentioned—namely, a rose-red anthocyanin filling the upper half or two-thirds of all the pigmented cells and a blue alkaline anthocyanin deposited in only a limited number of these cells. The cells destitute of this latter pigment are in small clusters of two to five and do not make up more than one-tenth of the colored areas of the pigment layer. Here, also, a mottling of the seed coat would result were it not obscured by the intense color obtained from the heavy pigmentation of this layer. In the basal-color layer we come to a variation that has not previously been observed. Portions of this layer underlying the heavily pigmented palisade cells, which give the black areas to the seed coat, are very heavily loaded with a dense yellow granular pigment, but where the basal-color layer underlies unpigmented or cream-white areas this pigment is either wholly lacking or consists of a mere trace. Where this pigment is present in large quantities it is massed in the upper cells of the layer and is a decided copper color, whereas the lower cells are, when the pigment is present at all, a pale lemon yellow.

A still more interesting departure from normal structure is found in the white areas of the palisade layer. In the black areas these cells are quite regular, both in form and in the gradual tapering of the cell cavity. But in the white or cream-white areas, although most of them are approximately regular, occasional cells, two or three together, show the strongly contorted form and the erratic spreading of the cell cavity, which were previously noticed in the albino cowpeas. In other words, in these cream-colored areas we find a duplication of the structure of the cells, as well as of color, that was discovered in certain cream-colored cowpeas previously described. Roughly estimated, 10 per cent of the cells of the white areas of this hybrid cowpea show this striking contortion in form and erratic spreading of the cell cavity. There is therefore seen to be a very strong contrast in structure, as well as in color, between the black and the white areas of this variety. This hybrid is the product of a Watson No. 5 crossed upon a Taylor No. 14, third generation.

Cowpea No. 227–5–1Re–17 has a color scheme of the Watson type. It is a pale-buff basal color, irregularly streaked with a purple black. The colored palisade cells are extremely dense with pigment, often appearing almost black. The quantity, however, varies greatly, some cells being practically pigment-free. This results in an irregular coloration of the seed coat. The pigment is an intense indigo-blue alkaline anthocyanin, with no trace of the rose-colored acid anthocyanin. There is also no melaninlike pigment in this layer. The cells are extremely irregular, the amount of contortion being greater than that of any variety previously mentioned. The cell cavity is also irregular, being
broadly flared out at the base, rapidly narrowing in the middle of the cell to a mere thread, and again broadening at the upper end. This irregularity of form should be noted in comparison with the same condition mentioned under other cowpeas belonging to this Watson type. In the basal-color layer large crystalline masses of a heavy melanin-like pigment are deposited in clustered cells along its upper stratum. Many of the cells are so scantily supplied with pigment as to appear practically colorless. The form of the cells is also more irregular than usual, showing a decided crumpling and contortion, even when expanded by means of caustic potash. This hybrid is the result of a cross of Sport No. 5 upon Coffee No. 27, third generation.

In Sport No. 5 the seed coat varies from cream white thinly spread over with purplish black spots arranged on the Watson type to individuals in which the purplish black pigment is so abundant as to give a dull purple-gray tone to the entire seed. All the cowpeas of this variety have a strong purplish black "eye." In the darker individuals the pigmented palisade cells, about one-fifth of the entire number, owe their color to an intense indigo-blue alkaline anthocyanin uniformly found in the lower third of the cells. The other four-fifths of the cells seem to be destitute of pigmentation. The somewhat purple tint of the seed coat would lead to the expectation that an acid anthocyanin would be associated with this blue anthocyanin, but no such color was discoverable. No trace of melanin-like pigment could be found in the palisade cells. Their shape is also significant. They are usually short, with thick walls, and display to a moderate degree the strange contortion in outline and twisting on their long axis referred to in the case of some other cowpeas. The basal-color layer is a pale or dirty lemon yellow.

The only difference discoverable between the light and the dark colored individuals is the greater infrequency of pigmented cells in the palisade layer of the former, there being only 1 in 20 having a trace of the blue-black anthocyanin above noted. It is also in smaller quantity. A mere hint of rose-red anthocyanin seems to be discoverable in palisade cells of the lighter individuals, but this is too uncertain to warrant a definite statement. The cells are also unusually short, thick-walled, and contorted in outline. The basal-color layer is the same as in the other form. No accurate data as to parentage of this variety were obtainable. It was originally secured from Mr. J. W. Trinkle, of Madison, Ind., but correspondence has failed to give the facts regarding its origin.

No. 220–2–2Re, another Watson type, has a pale-buff seed coat irregularly streaked with dull purple. The pigment is intensified around the hilum, producing what is known as an "eye." A minute amount of rose-red pigment is found in the upper part of the palisade cells. It quickly dissolves in water, leaving a dense mass of granular indigo pigment in the extreme basal end of the cell cavity. A
large part of the cells, however, are practically free from any color, this being the cause of the very irregular streaked appearance of the seed coat. The cells are extremely irregular in shape, which gives to a section through the palisade layer a marked unevenness of appearance. The basal-color layer has a pale dull-yellow pigment massed in its lower cells. A good number of the cells seem to be colorless. The variety is the second generation of a cross of a Watson No. 5 on a Coffee No. 16.

One other cowpea needs to be mentioned, No. 0618. It is pale buff or clay, dusted over with brown gray on the Watson pattern. It is further pigmented with very small, round, deep, purple-brown dots, similar to the color massed around the "eye." The pigmentation of the palisade cells is confined to a little over half their number, the pigment being in the lower third of the cell cavity. It is a blue alkaline anthocyanin, with little or no trace of the acid form of this pigment, although in the minute areas represented by the deep purple-brown dots of the seed coat a small amount of red acid anthocyanin seems to be present. All the palisade cells are strongly contorted. The underlying basal layer is narrow and also strongly contorted. It is largely destitute of pigment, except for segregated masses of a deep-orange color located in widely separated groups of cells in the upper portion of the layer. The parentage of this hybrid is unknown. Like No. 5, previously mentioned, it was obtained from Mr. J. W. Trinkle, of Madison, Ind., but correspondence has failed to give any data regarding its origin.

III.—COWPEAS HAVING ONLY A MELANIN-LIKE PIGMENT IN THE PALISADE LAYER

The third class of cowpeas—namely, those in which a melanin-like pigment alone is found in the palisade layer—ranges through various shades of light brown, buff, and red.

The first of these is a pale-buff or clay-colored cowpea, No. 237-3-7. A minute quantity of melanin-like pigment was detected in the palisade cells, and that only in the case of darker specimens. The very pale buff-colored seeds show no trace of pigmentation in this layer. The effect of this minute trace of pigment on the general color scheme of the seed coat must be small. Indeed, the color is easily explained by the stronger pigmentation of the basal-color layer. This layer is a vivid brownish yellow color. All the tests for anthocyanin failed to show a trace of this prevailing pigment in any cells of this cowpea.

It should be noted that the presence of melanin-like pigment in the palisade cells is of some interest in regard to the affinities of this cowpea to others in which it is also found, its parentage being Red No. 4 crossed on Taylor No. 14, second generation from the cross.

Another practically monochrome cowpea is No. 27544, known as the Iron cowpea. It ranges from a delicate buff or clay to a strong reddish
brown with an intensification of color about the hilum. A part of the palisade cells, perhaps two-thirds to four-fifths, contains a moderate quantity of the melanin-like pigment, the remaining cells being pigmented to only an extremely slight degree. The pigment is scattered throughout the cell cavity in a fine granular condition, instead of being massed in the lower end, as is usually the case. The basal-color layer ranges from a strong yellow to a decided copper or orange color, varying in this respect according to the general coloration of the seed coat itself. It has been impossible to learn the parentage of this well-known and widely cultivated variety.

A cowpea strongly marked in what is known as the Whippoorwill pattern, made up from a basal color of a pale clay heavily marbled with a rich reddish brown, is No. 242–3–1. The palisade cells show the variation in coloration that would be expected by the marbled character of the seed coat. The strongly pigmented cells of the marbled areas are a rich reddish brown, approaching to the color found in the basal-color layer. The other cells, making up the unmarbled areas, though not actually destitute of pigmentation, contain so minute a quantity as to only slightly affect the color of the basal layer beneath it. This latter layer is of an intense copper tint, the pigment being deposited in dense masses in the upper part of the layer. A very unequal distribution of the pigment in this layer corresponds somewhat but not accurately to the unequal distribution of the pigmentation in the overlying palisade layer. No trace of anthocyanin was found in any of the cells of this cowpea. Although the optical effect in the matter of color is not involved in the presence of tannin, it may be stated that this substance is more abundant than usual in this particular cowpea. Its parentage is Clay No. 17, crossed on Whippoorwill No. 6, second generation.

No. 243–5–3 is a variety with monochrome seed coat ranging in color from a light to a very dark reddish brown. The cells of the palisade layer show a strong granular pigment of a light red, in some cases almost brick red, quite uniformly massed in the extreme lower end of these cells. In some instances the pigment is so finely divided that it is difficult to discover it except when masses of cells are superimposed upon one another. Although the seed coat gives no indication of an unequal distribution of color, the sections seem to indicate that there is a slight excess of pigment in certain groups of cells over that in cells surrounding them. The basal-color layer has a much lighter tint than that found in the cowpea last mentioned. It is a lemon-yellow color, intensified in darker individuals to a decided brassy tone. The form of the palisade cells is normal. The parentage of this cowpea is a Red No. 4 crossed on a Whippoorwill No. 6, second generation.

No. 242–5–2 has one of the two parents last mentioned and is similar in general color scheme, varying from buff to reddish brown. The palisade cells are abundantly supplied with a dull-yellow pigment, but quite vari-
able in quantity. In view of the fact that one of its parents is a Whippoorwill this unevenness of distribution of pigment in the palisade layer is significant. The cells of this layer are longer than usual and the taper of the cell cavity is somewhat sudden and blunt. There is, however, no contortion. There is no evidence of anthocyanin. The color in the basal-color layer is somewhat different in tone from that in the palisade layer, being a more vivid yellow, approaching orange; but both give reactions that indicate the pigment to be the usual melanin-like substance. Tests for tannin show that the basal-color layer is highly impregnated with this substance. The variety is a cross of Whippoorwill No. 6 on Clay No. 17.

No. 216-6–4, a light-coffee cowpea obscurely streaked, shows the basal-color layer to be a vivid yellow, while the palisade layer is buff to brown and quite variable in degree of pigmentation. The pigment in both is a strongly granulated melanin-like substance. There is no trace of anthocyanin. The cowpea is a second-generation hybrid produced by crossing Red No. 4 upon Coffee No. 16.

No. 216–1–7 is a light to dark coffee cowpea. Closely observed, the seed coat shows a slight tendency to mottling. The very decided color of the seed coat would lead one to expect a heavy pigmentation in the palisade layer, but such is not the case. It is pale reddish brown, and not only is comparatively light, but treatment with various reagents fails to produce much intensification. In the basal-color layer the pigment is far more abundant and is confined to three or four layers, where it is somewhat unevenly distributed. It seems that the deep red brown of this cowpea is due to the pale reddish brown of the palisade layer plus the intense orange yellow of the basal layer. The parents are Red No. 4 crossed upon Coffee No. 16; in other words, it is identical with those of the cowpea last mentioned, the variety examined being the second generation of this cross.

A most interesting cowpea, known as Old Man, bears the Government number 17354. It has a cream-white seed coat obscurely and faintly streaked with yellow brown. The deeper color is very strongly deposited about the hilum, so that its character can there be readily tested. Transverse sections of the seed coat show that in almost all instances the palisade cells are practically destitute of pigment. However, a minute quantity may be detected by very close observation, and it is observable that this is highly variable, even within the narrow limits just mentioned. In other words, it corresponds to the very obscure streaking of the seed coat itself. It is of a melanin-like character without any admixture of anthocyanin. The palisade structure is decidedly abnormal, its cells being much wider in proportion to their length than common, enormously contorted, and the unusual twisting upon the long axis is here carried to an extreme. The whole palisade layer is loosely put together with abundant intercellular spaces. The basal-color layer has an exceedingly meager
and pale representation of the pigment usually present. The pale-cream color of this cowpea is doubtless due to the small quantity of melanin-like pigment diffused through the basal-color layer, and the streaked and indistinct marking of the seed coat is caused by the minute quantity of the same pigment unevenly distributed in the palisade layer. It is interesting to note that the palisade cells in the neighborhood of the hilum, where the color is quite intense and forms what is known as the "eye," are very much larger than on the rest of the seed coat and almost entirely free from the contortion and twisting already mentioned. In other words, the irregularity of form seems to be directly connected with the white or cream-white character of the seed coat. This same remarkable parallel has already been noted in several other cowpeas. The basal-color layer in the neighborhood of the hilum is very heavily charged with a melanin-like pigment, but there is here a somewhat unusual arrangement in that the lower cells of this layer are of a somewhat pale lemon yellow, while the separated masses in the upper part of the layer are a deep orange or orange buff. The reactions of these two, however, are identical. No information has been obtained as to the parentage of this variety.

IV.—COWPEAS HAVING BOTH A MELANIN-LIKE PIGMENT AND ANTHOCYANIN IN THE PALISADE LAYER

The fourth class includes all cowpeas showing both anthocyanin and melanin-like pigment in the palisade layer. The first one to be mentioned, No. 214-5-10, is generally described as having buff markings upon a black ground. The fact is that it is a cowpea with a strong buff basal color almost covered with large black areas. In other words, the black is superimposed upon the buff and not the buff upon the black. The two colorations of the seed coat are accompanied by a quite different condition of the palisade layer. A melanin-like substance is to be found in all pigment cells of the seed coat both in the buff and in the black areas. An acid anthocyanin is present in all the palisade cells of the black areas, but in no case in those of the buff areas. An alkaline anthocyanin is to be found in one-half to three-fourths of the palisade cells of the black areas, but in none of the cells of the buff areas. In all cases the alkaline anthocyanin is massed in the lower end of the cell cavity and the acid anthocyanin occupies principally, if not wholly, the upper half of the cell cavity. The color produced by these two anthocyanin pigments is a more intense purple than has been found in any other cowpea, and when the rose colored acid anthocyanin is extracted, the indigo-blue or alkaline anthocyanin found in one-half to three-fourths of the cells of the black areas is larger in quantity and more vivid in color than is generally the case. In the buff areas there is evidently neither of these phases of anthocyanin. These cells are, however, pigmented with the melanin-like material found in other cowpeas. A comparison of the form of the palisade cells in the two areas is also of interest. Those in the black
areas are unusually symmetrical, so much so as to attract attention, but in the buff areas there is a slight tendency to contortion and a more unequal tapering of the cell cavity. In other words, there is a hint in these cells of the abnormality of form found in a high degree in some other cowpeas. The basal-color layer is well supplied with the usual yellow melanin-like pigment in all parts of the seed coat. The parentage of this cowpea is White No. 7 crossed upon Black No. 22, it being the second generation hybrid.

A cowpea that appears in general purplish black, but somewhat unevenly colored, is No. 201-1-2-9. A study of its seed coat makes the cause of this evident. Many of the palisade cells contain only one anthocyanin pigment—namely, a strong rose purple. This dissolves rapidly in water, leaving the cells colorless. In some cases a second color remains in the cells and proves to be minute particles of the usual melanin-like pigment. In addition to the foregoing a number of the cells contain in the lower end a strong deposit of blue alkaline anthocyanin. This is more clearly seen after the extraction of the rose-colored anthocyanin. The melanin-like pigment is unevenly distributed in the palisade layer, many of the cells being destitute of it, so that it is safe to state that in some areas of this cowpea this pigment is associated with both phases of anthocyanin while in other parts we have either the rose anthocyanin alone or the rose and the indigo-blue phases of this pigment without the presence of the melanin-like pigment. The cross producing this variety is Black No. 13 upon Blackeye No. 19, being the third generation from the cross.

Although No. 239-5-3-18 was referred to under the first division as being an essentially cream-white cowpea, the strong purple eye of this variety places it in this last division; for by making transverse sections in the neighborhood of the hilum where the pigmentation is intense we find that the palisade layer contains both the acid and the alkaline phase of anthocyanin associated with the melanin-like pigment. The rose, acid anthocyanin is quite generally present in these pigmented cells, but a large number of them, perhaps two-thirds, are destitute of alkaline anthocyanin. The basal-color layer is abundantly colored with the usual orange-yellow pigment. As stated, the palisade cells in the cream-white seed coat, which constitutes almost the entire surface of this cowpea, are unusually irregular in form. It is therefore quite interesting to see that the strongly pigmented palisade cells in the neighborhood of the hilum show no trace whatever of these irregularities. As already stated, the hybrid is the third generation of a cross between Watson No. 5 and Taylor No. 14.

A cowpea having a basal color ranging from pale buff to strong red brown and very heavily spotted with black is No. 214-6-7-2. There is seen to be a strong brassy yellow pigment in the palisade cells. The basal-color layer is usually densely filled with the same colored pigment,
but where the basal color is pale buff instead of red brown it is very
deficient. The deeper tint of the basal color in some seeds is therefore
due, at least in part, to a greater quantity of pigment in the basal-color
layer rather than to any difference in the pigmentation of the palisade
layer. This is the reverse of what is usually found in cowpeas of variable
tint, their difference usually being brought about by variation in the
degree of the pigments of the palisade cells superimposed upon a
uniformly pigmented basal layer. The black areas are due to a dense
blue alkaline anthocyanin confined to the lower third of the cavity of
the pigmented cells and so heavily deposited that only long action by
different reagents brings about the usual changes. The brassy yellow
pigment also contained in the palisade cells gives the usual reactions.
It is strongly granular and in unusually large quantity. It is well to note
the presence in the same cells of these two forms of pigment in con-
nection with the fact of the very slow response of the anthocyanin to
the usual reagents, as this behavior will be commented upon in other
cases. The form of the palisade cells is slightly irregular, but not
exceedingly so, and this is confined almost entirely to the lighter portions
of the seed coat. The parentage is White No. 7 crossed upon Black
No. 22, second generation.

Cowpea No. 14 has a pale-buff to red-brown basal color, strongly
speckled with black spots on what is known as the Taylor pattern. In
individuals of the lighter basal color we find that the palisade cells in
the ground-color areas are so nearly destitute of pigment that it is diffi-
cult to discover its presence. The basal-color layer is also a dirty
yellowish brown instead of the stronger brown that would be expected.
The areas spotted with black owe this color to an intense blue antho-
cyanin in the lower half of the cavity of the palisade cells, the proportion
of these to uncolored cells being about as 1 to 5. The anthocyanin
extends up in these cells much higher than in most cases, sometimes
reaching the upper end of the cavity. In individuals having the darker
ground color, the presence of melanin-like material in the palisade cells
is very evident and the basal-color layer is seen to be much more strongly
tinted with a strong copper-colored melanin-like pigment. In this cowpea
we again find that only by long treatment will the usual reagents bring
about the expected reactions on the anthocyanin. Caustic soda, hydro-
chloric acid, chloral hydrate, etc., are very sluggish in the changes
produced, so that there seems to be an impediment in the way of their
reacting upon this sensitive material. The palisade cells in both dark
and light varieties are long, narrow, evenly tapered, and symmetrical.
No data have been secured as to the parentage of this variety.

No. 237–3–2 also ranges from pale buff or clay to strong red brown
and is speckled with black. The black color is due to a blue alkaline
anthocyanin deposited as usual in the lower end of the palisade cells,
and in this case also the reagents are extremely slow in producing results
upon this pigment. This fact is of interest when taken in connection
with the fact found in the other cowpeas above mentioned that it is
intimately associated in these cells with a very large amount of melanin-
like pigment. In what way the intimate mixing of these two protects
the anthocyanin from the rapid effect of reagents it is impossible to
say, but it seems probable that some such interference is brought about.
The melanin-like pigment is coarsely granular and orange brown in color.
It is also to be observed that this pigment modifies the color tone tardily
secured by the reactions of various reagents. Thus, with hydrochloric
acid, the blue anthocyanin does not give a rose color, but rather a deep
cherry red, probably due to the mixture of the usual rose tint with the
orange yellow tint of the melanin-like pigment associated with it. The
speckling, which is of the Taylor type, is due to anthocyanin contained
in certain palisade cells. The basal-color layer has the usual pigment.
The cowpea is the product of a cross of Red No. 4 upon Taylor No. 14,
second generation.

Another cowpea of a different color scheme needs mentioning, No.
243-6-1. This one ranges from pale buff to strong red brown, speckled
with black, on the New Era pattern. The palisade cells which repre-
sent the ground color—that is, which are not connected with the speck-
ling—are, as in the former case, of a dull brassy yellow. The same tint
is found in the basal-color layer. From one-third to one-fifth of the
palisade cells contain a deep-blue alkaline anthocyanin located in the
lower end, and here again it was discovered that all the reactions normal
to this pigment are greatly delayed, so that a longer period of time is
needed to make the necessary tests. The melanin-like pigment is present
in large quantity in all the palisade cells. This variety is the second
generation of a cross of Red No. 4, crossed upon Whippoorwill No. 6.²

We come now to a cowpea which is probably wild. It is a Vigna
sinensis (?), having the number 01653, and comes from Sokoto Province,
Upper Nigeria, Africa. In some respects it is quite different from the
cultivated cowpea. In matter of size it is from one-seventh to one-
eighth the average size of cultivated varieties. Its markings are
extremely interesting, in that they display on the same seed coat all of
the features which are found to make up the color schemes of the culti-
vated cowpeas, not only all the colors but all the styles of distribution.
First, there is a basal color which ranges from pale clay or buff to reddish
brown; second, this is extensively blotched or marbled with deep brown
red, sometimes pretty well covering the seed coat; third, there is present
a fine speckling of blue-black dots scattered over the seed coat; and

¹This intimate mixing of blue anthocyanin with a deep-tinted melanin-like pigment and the consequent
resistance of the former to reagents misled the writer at first into concluding that he here had to do with a
black melanin-like substance; and in some remarks before the Washington Botanical Society on May 7,
1912, the writer included such pigment with the others found in the cowpea. A report of this meeting,
in Science, June 28, 1912, also contains this error, which is now corrected.

²Some individuals of this variety proved to have been contaminated by crossing; hence, the presence of
speckling in some of its descendants.—W. J. Spillman.
fourth, there are occasional spots in the form of large roundish intensely black areas. Transverse sections show that the general structure of the seed coat is identical with that of cultivated varieties. The palisade cells are of the same general shape and are as to size in the usual proportion to the rest of the seed coat. The underlying layer of so-called hourglass cells is also the same and is, as elsewhere, empty. Beneath this is the usual basal-color layer, supplied with the regular orange or yellow melanin-like pigment. The red areas of the seed coat overlying the basal clay or buff owe their color to a strong orange or red-brown pigment in the palisade cells, identical in organization and in its reactions with the similar color in cultivated varieties; in other words, a melanin-like pigment. The fine speckling is in this case also due to an intense blue anthocyanin pigment in the lower end of certain palisade cells and it is also here associated with the melanin-like pigment mentioned under former headings, and, as in the other cases, it responds very slowly to the reaction of reagents. Furthermore, the areas represented in the seed coat by large black spots contain both red acid anthocyanin and blue alkaline anthocyanin, as is the case in the black areas of cultivated cowpeas. The complete uniformity of methods of coloration, as well as of schemes or patterns of coloration in this supposedly wild cowpea, when compared to our cultivated varieties, is of considerable interest. There is no trace of distortion or irregularity in the palisade layer. Of course, no knowledge is obtainable as to its origin. It was received from Kew Herbarium and was collected by J. M. Dalziel.

SUMMARY

The greatly diversified color schemes of the different varieties of cowpeas may therefore be reduced to two factors: (1) An extremely uniform basal color, ranging from very pale yellow to deep copper red, but found to be in all cases due to a melanin-like pigment deposited in the basal-color layer, the differences in tint being unquestionably caused by differences in quantity rather than in character of the pigment present; and (2) a superimposition upon this basal color of variously arranged pigment areas in the palisade layer, the outer layer of the seed coat, the pigments here being of only two kinds, first, a melanin-like pigment very generally identical in color and behavior to that found in the basal layer, and, second, an anthocyanin pigment, either associated with this or found in separate cells. And further, this anthocyanin pigment may be of a red color, on account of an acid condition, thereby producing various shades of purple and rose; or it may be alkaline in character, thereby producing various shades of blue and black, and these two may be found in the same cells or in some instances in separate cells. Finally, according as only one, or more than one, or all of these pigments sometimes found in the palisade layer are actually present there, and according as they are uniformly distributed throughout its cells or are variously localized in large or small
areas of its cells, do we get the remarkably diversified blotching, streaking, speckling, marbling, or monochrome colorations which characterize the different varieties of cowpeas.

A word should be said regarding the very interesting cases of distortion in the palisade cells mentioned under some of the foregoing varieties. Referring to the facts there mentioned, it will be seen that where the seed coat of the cowpea is white or cream white, as in Nos. 0362 or 17354, or where it has a certain white area, as in Holstein No. 239-4-3-6 or in No. 239-5-3-18, or even in cases where there is merely a light speckling or dusting over of this cream-white color, as in Sport No. 5, in varieties of the Watson type, as No. 227-5-1 Re-17, in certain individuals of No. 17354, and in No. 0618, the palisade cells show great distortion of outline and unevenness in the cell cavity. Furthermore, in most particolored cowpeas of strongly contrasted tints, such as Holstein No. 239-4-3-6, or the black eye in No. 239-5-3-18, or the coffee-colored eye in No. 17354, the strongly colored areas have perfectly regular, symmetrical palisade cells, while the lighter areas are more or less strongly contorted in form and irregular in the cell cavity. In other words, there is traceable in all of these cowpeas a decided correlation between the morphology of the palisade cells and the suppression of the pigments in these cells.

PLATE VI

Fig. 1.—Transverse section of the seed coat of a cowpea, similar to that shown in text figure 1, but showing the relative thickness of three layers, as on the seed. The cells are not expanded with chloral hydrate. cu, Cuticle; a, palisade layer; b, middle or hour-glass layer; c, basal-color layer. Somewhat diagrammatic.

Fig. 2.—Seeds of cowpeas, showing some of the variations in the style of marking of the seed coat. Natural size.