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THE COLORATION OF ANTHER AND CORBICULAR POLLEN

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Recently interest has been centered in bee literature upon pollen, its use by the honey-bee, appropriate substitutes for pollen during a dearth of that food item of the honey-bee, and the importance of the honey-bee in cross-pollination in agriculture.

Literature on pollen is quite diffuse. In the United States excellent work in pollen research has been made by such men as Young (1908) and Wodehouse (1935). Some pollen studies concern the morphology or external structure of the pollen grains, some are directed to their anatomy or internal structure, others consider the chemical constituents of pollen, pollen as a cause of hay-fever, etc.

Most of the pollen studies give little or no notice to the coloration of the pollen grains themselves, and yet it would seem to be one of the important points overlooked. Todd (1941) states that, "The color of pollen is of great economic importance to a beekeeper. Some of the coloring materials are fat-soluble in beeswax. They are the source of the yellow color in beeswax. Some pollens also contain water soluble coloring materials, and they appear to be responsible for the amber color of honey. The fresh nectars so far examined, even from plants that produce dark honey, appear to be colorless." Fischer (1890) one of the great recent investigators of pollen was one of the first to note the importance of pollen color to the student of pollen morphology. Related species of pollen are quite often similar and sometimes the species of a genus can hardly be distinguished from each other except by their color. Erdtman (1933) specifies that for a complete analysis of pollen forms in research, notes on their color are also desirable.

Early writers on pollen, such as Grew, Malpighi and others remarked about the coloration of pollen grains and gave some specific identifications. In the 19th century Francis Bauer gave some sketches of pollen with a word or two added about the color. More recently Armbruster and Jacobs (1929) and Oenike (1934–5) give notice to the color of pollen grains but presumably as seen under magnification. Wodehouse (1935) in his monumental work on pollen grains, dismisses the entire subject of coloration with a few introductory words on "the yellow powder" and there is no further reference to pollen colors in his extensive descriptions and classifications. Microscopical journals, the beekeeping journals and a few other writings make an occasional reference to the color of individual species of pollen, but no comprehensive study has been made of this subject.

CORBICULAR POLLEN

It should be noted at once that all color appraisals or emphasis on the importance of pollen color so far (except that of Todd) are for anther pollen, i. e., either the color of the pollen masses on the anthers of flowers or of the individual pollen grains examined. The color of pollen, however, after it has been gathered by the

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honey-bee and as it is seen by the beekeeper or other observer in the form of pellets being brought into the hive is somewhat different. This type of pollen the author will refer to hereafter as corbicular pollen, and this paper refers to the masses of anther pollen and of corbicular pollen and not to individual pollen grains. word "corbicular" is not to be found in Webster's New International Dictionary (1942) but was used some years ago by Casteel (1912) in describing the process of pollen collecting by the honey-bee. Most naturalists and poets limit the color range of corbicular pollen, referring to it simply as "the golden dust" of the flowers, or the "golden grist" which the bees bring home, or it is spoken of as "the golden foam of pollen." Bee literature itself abounds in generalizations. The color of pollen is said to range from "cream to dark purple" or it may be "all shades, from black to white and many hues of the rainbow." Less frequently are "yellow, orange, red, brown or green" specified. Cheshire (1888) says that the returning bees bring back to the hive "colored material . . . some shade of yellow or orange . . . crimson, green and even black . . ." His further references are to the color of the anther pollen of the Rosebay Willow herb and of the Purple Loosestrife not to corbicular pollen. Sempers (1911) lists for the locality of Aikin, Md., 39 plants and 13 colors. Some of these colors seem to be for anther pollen, as "white for Boneset," "greenish white" for the Pear, while "brown" for Wintercress and White Clover seems to refer to corbicular pollen. Later (1912) he specifies corbicular pollen when he states that the honey-bee gathering pollen on the Wild Carrot formed "greenish-white pellets" to take home. Bisson and Vansell (1940) investigating the properties of beeswax list twenty-one plants and fifteen natural colors of the pollen. The reference seems to be to the anther pollen. Vansell (1942) describing pollination factors lists twenty-two plants and gives fifteen colors for the corbicular pollen and adds other load characteristics. Todd and Bretherick (1942) describing the composition of pollen, list thirty plant pollens. Of these six are anther pollens ("hand collected") arranged in five color categories and twenty-four are corbicular pollens ("from the bees") arranged in fourteen color categories. An annual feature of the British "Bee Keeping Annual" (1944) is the listing of forty-three English pollen plants and their arrangement in eleven color classes.

In none of the above writings however was any notice given to the factor of change of coloration. There is a definite need to distinguish between anther pollen color and corbicular pollen color. This paper intends to show that there is an appreciable difference in color between anther and corbicular colors of pollen.

COLOR

Color may have a variety of meanings. While color and hue are used more or less synonymously, in this paper it will be evident that color refers to the general classes of sensations evoked in the nervous system of the eye and hue is a more specific identification of the same. Coloration simply describes the pollen's state of being colored.

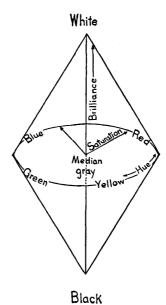
There are two classes of colors; the chromatic colors, as reds, yellows, greens and blues, and the achromatic colors, as white and black and the intermediate greys. The latter have no hue and differ from each other only to the extent of their difference or resemblance to white or black. The achromatic colors may be arranged in a scale from black to white with all the intermediate greys between. Median grey at the center would differ equally from black and white and other greys according to their sense differences. This series can be made into a scale by assigning ordinal numerals to each ascending grey from black as zero (0) through median grey, equal to four (4), on to white as eight (8). The same scale may be expressed in terms of percentages. The relationship expressed on this scale is called brilliancy. Thus, black or zero brilliancy; 1 or 12.5%, very low brill.; 2 or 25%, low brill.; 4 or 50% brilliancy; 6 or 75% brill.; 7 or 87.5% brill.; and so on

with 8 or 100% brilliancy. The extremes or ideal limits are not realized in practice. The Munsell system uses nine degrees of brilliance, called "value."

Hue is the attribute in respect to which we describe colors as red, yellow, green or blue or any intermediate between two of these. Arranged in a circle they form a color circle. All hues may be described in terms of the four psychologically primary hues red, yellow, green and blue. Red-yellow equally resembles and differs from red and yellow. Between each consecutive pair of primary hues many intermediates may be recognized. Thus arbitrary points of division can be established on the hue cycle, whence we have, red, yellowish-red, reddish red-yellow, red-yellow (midway between red and yellow), yellowish red-yellow, reddish-yellow, greenish yellow, etc. These steps represent equal visual and not physical intervals. The Munsell system uses ten principal hues and has one hundred hue steps in its color cycle.

Colors of the same hue and brilliancy may differ from each other in vividness of hue, that is in degree of difference from grey. This attribute is called saturation. In terms of these three attributes colors can be arranged in a tridimensional space or color solid, as Fig. 1. Two equal cones are placed base to base. The achromatic

Fig. 1. Color Solid or Tridimensional Color Space. Hues are represented cyclically according to the hue cycle, on a plane perpendicular to the axis. The plane's level determines the brilliancy of the various hues. When close to the white apex colors are of great brilliancy and when close to the black point then they are of low brilliancy. Saturation is measured radially on each plane. Maximum saturation is at the rim and zero saturation is at the center.



white and black are represented by either apex, and the grey series of hues are along the vertical axis with median grey in the center. The vertical axis represents brilliancy. Corresponding to each degree of brilliancy is a plane perpendicular to the vertical axis and in this plane lie all the colors of equal brilliance. Hue is represented in each plane cyclically in the order of the color circle. Saturation is represented radially, with the axial points (greys) as reference points of zero saturation. The closer a hue is to the vertical axis, the less its saturation. Hues on the outer edge of the circle have the highest saturation. A scale of saturation similar to that of brilliancy above may be used.

To express hue, brilliancy and saturation, numerals may be used and they are referred to as color constants or dimensions. This author has used the ordinals (or the percentage values) only to represent saturation and brilliancy. To represent hue the author has used the colorimetric values of the various hues as expressed in wave lengths in millimicrons $(m\mu)$. These wave lengths and the scale values of saturation and brilliancy may be called the physical correlates.

COLOR VALUES

The values of the psychologically primary hues, referred to hereafter in the tables are the mathematical mean of the color range as given in The Handbook of Colorimetry (1936), i. e., red, 655 millimicrons ($m\mu$); yellow, 580 $m\mu$; green, 535 $m\mu$; and blue 475 $m\mu$. The values of the intermediates are taken from Judd's (1940) listing, as, yellow-red, 598 $m\mu$; green-yellow, 566 $m\mu$; and blue-green, 495 $m\mu$. For yellowish-red, (English vermillion) 644 $m\mu$; for reddish red-yellow, (Mineral Orange) 614 $m\mu$; and for reddish-yellow (light Chrome yellow) 585 $m\mu$, Rood's (1942) values were taken. The 591 $m\mu$ for yellowish yellow-red (Medium gold) is from Nutting (1931), and the Handbook of Colorimetry (1936) supplied the values of yellowish-green (Olive green) 572 $m\mu$; and of yellowish yellow-green (Apple green) 568 $m\mu$. The values of the greenish yellow-green hue, 550 $m\mu$, and of yellowish green 540 $m\mu$ are interpolations of this author.

COLOR MATCHING

This entire study it should be noted is not one of color analysis but of colormatching. No spectroradiometer or spectrophotometer was used. The method of color-matching used by the author is the one suggested in A Dictionary of Color (1930). This volume contains reproductions of about 7,000 different colors, but plates numbered 1 to 24 showing the spectral colors from Red to Orange, Orange to Yellow and Yellow to Green (about 2,000 in all) were the most useful. A sheet of neutral grey paper having a reflection factor of about 42.9% was used as a mask to bring a single color into immediate focus. An opening of 9 square millimeters reduced the area of the hue "samples" to a minimum. The color mask was used at all times to check the individual anther or corbicular pollen masses. A linen tester with a focal length of 50 millimeters giving about four magnifications (4×) aided in checking the pollen masses. The illumination used was that of natural daylight on such days as might be termed clear and sunny. Direct sunlight was never used. Most pollens were rechecked within doors under a window having northern exposure, but showed no difference. The color charts of Webster's New International Dictionary (1942) were used to a limited extent and those colors are identified on the list of Table I by the lower case letter "w." Not every hue or color has a name, hence some are referred to only by Plate number, by the column letter and by the row number, as, 9 E 2. At times a closely related color is suggested for the unnamed hues. This is indicated by the symbol \approx . The author believes he is gifted with normal color vision and the maximum time required for each pollen color check was about twenty minutes.

FIELD METHODS

During the spring and summer seasons of 1944 and 1945 visits were made afield in the New York Metropolitan district and to the Brooklyn and New York Botanical Gardens for anther and corbicular pollen specimens. The characteristic movements, as described by Casteel (1912) and the tell-tale results in the corbiculae of the honey-bee served to distinguish and identify the pollen gatherers from the nectar gathering honey-bee. Bees with fair to large amounts of pollen showing in their corbiculae were seized with a special bee-catcher (Fig. 2) and destroyed with cyanogas in a few seconds. For each specimen a minimum of nine honey-bees were taken. Since Alfonsus (1933) warns that at times drops of propolis may be mistaken for pollen from a distance all specimens were examined under the microscope and identified as pollen. Under more favorable circumstances fifteen to thirty minutes sufficed to obtain the necessary specimens of an individual plant species. Under less favorable circumstances, due to atmospheric conditions affecting dehiscence of the anthers, a much longer time was needed. Shedding of pollen was heavier in the morning and in the late afternoon. Other insects such as bumble-bees, solitary wasps, flies and countless thrips competed with the honey-bee in gathering or consuming the pollen. During a general

scarcity of the much-needed pollen an occasional bee was found to work from one kind of flower to that of an entirely different species of flower while grathering pollen. Sladin (1912) in England too observed this occasional mixing of pollen by the honey-bee on a single trip. But that is only by way of exception. Bees show great constancy to a particular kind of flower even though a similar species is near at hand.

Anther pollen was generally checked for color in the field, but never in direct sunlight. Pollen masses large enough to conceal a differently colored pollen sac were removed in entirety, checked against the color chart, and the species and color recorded. Several specimens were used in each instance but no variations were noted. It should be noted, however, that Purple Loosestrife is trimorphus and the different length anthers carry different colored pollen. The Wand Lythrum, Lythrum virgatum, of the Brooklyn Botanic Garden, had long anthers with Charteuse colored pollen and short anthers with a Chrome Primrose colored pollen. Corbicular pollen of the bees however was all Yew green. Purple Loosestrife, L. salicaria, found near Princeton Junction, N. J., however, had long anthers with Bottle green colored pollen and shorter anthers with Chrome Primrose colored pollen. When pollen was scarce or overshadowed by a differently colored pollen sac, an appreciable mass was teased loose and gathered with a probing needle and this mass then checked for color.

The corbicular pollen was checked for color as soon as possible, at least before there was any danger of the pollen becoming "dry." The entire hind leg of the bee

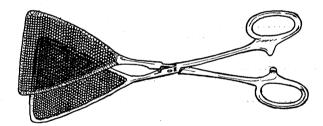


Fig. 2. A bee-catcher. The nine-inch instrument has two triangular spring wire (No. 11 gauge) frames brazed to an arterial clamp. These frames are covered on the outer side with tulle or 13 mesh cotton netting. When closed there is a bee-space (0.25 inch) between the pieces of netting. On smaller flowers the pollen collecting honey-bee and the flower are both grasped together or enclosed between the netted frames, on larger flowers the bee is swept up between the lateral edges of the bee-catcher. The enclosed bee is then held over a cyanogas bottle until destroyed. Between the finger holes is a ratchet locking the device.

was removed, graded for uniformity of color and size of the pollen pellet. With a thumb forceps they were matched for color against the squares of the color charts. The pollen masses were then pried loose from the pollen-basket and rechecked. There was no variation in color as a result. The pollen masses were not, therefore, transparent nor were they influenced by the darker colored tibia of the honey-bee. Some pollen collectors working on a single species of flower showed slightly different colored masses of pollen in the corbiculae, that is, a few would have one color pollen and others a similar or entirely different hue. Parker (1922) also noticed this dissimilarity of color in the pollen masses gathered by the honey-bee from a single species of flower. Thus, he says, bees working on the Box Elder, Acer negundo, showed yellow-green corbicular pollen and some showed green pollen. For the Black Maple, Acer nigra, some bees had yellow and some had green-yellow pollen. On drying these pollen masses, only one color persisted and under the microscope all the pollen were pure. All "double" colored corbicular pollen reported in Table I was examined by the author and found to be pure.

The results of the Field Methods and the Color Matching are given in Table I. The flowers listed are entomophilous or insect pollinated. The first column lists the color class and approximate wave length in millimicrons, to which is added the specific hue of the anther pollen. The second column lists the plants examined and their scientific names according to the American Joint Committee on Horticultural Nomenclature (1923). The third column shows the color of the same pollen after it was gathered and packed by the honey-bee in its corbiculae. Two colors listed in this third column indicate that about one-half of the pollen collecting bees showed one colored pollen and about one-half showed the other colored pollen in their corbiculae.

TABLE I

FLORA ARRANGED ACCORDING TO THE COLOR OF THE ANTHER POLLEN AND SHOWING THE CHANGE OF COLOR EFFECTED WHEN THE POLLEN IS COLLECTED BY THE HONEY-BEE IN ITS CORBICULAE.

Anther Pollen Color. Class and Sp. Hue	Specimen	CORBICULAR POLLEN
YELLOWISH RED. 644 mµ Cherry red hue	Red Horsechestnut—Aesculus carnea	Raisin hue.
Coral red hue	Common Mignonette—Reseda odorata	Zanzibar and Rustic brown.
REDDISH RED YELLOW. 614 mµ Burnt orange hue	Wallace Dahurian Lily — Lilium daurianum wallacei	Chinook hue.
RED YELLOW. 598 mµ Bitter sweet orange hue	Horsechestnut—Aesculus hippo- castaneum	Anatolia.
Cadmium orange	Japanese Rose—Rosa multiflora (Hiawatha Rose—Rosa sp.) (Evangeline Rose—Rosa sp.). Yellow Hornpoppy—Glaucium flavum	Henna. Henna. Chestnut. Persian orange (w).
Cadmium yellow	Scotch Broom—Cylisus scoparius	Punjab.
YELLOWISH RED YELLOW. 591 mµ Deep chrome yellow	Winter Hazel—Corylopsis spicata Yoshino Cherry—Prunus yedoensis Cherry—Prunus (mahaleb) Dandelion—Taraxacum officinale Miyama Cherry—Pr. maximowicii Cypress Euphorbia—Euph.	Raw umber (w). Mandarinorange (w). Tan and Acorn (w). Terra cotta and Raw umber (w). Cocoa (w).
	cyparissas	Caledonian brown (w).
	White Dutch Clover—Trifolium repens	Caledonian brown (w).
	Cathay Japanese Rose—Rosa multi- flora cathayensis	Terra cotta (w).
	rhoes Prairie Rose—Rosa setigera	Khaki (w). Mandarin orange and Tan (w).
	Deutzia sp.—D. ningpoensis	Citrine. Chestnut. Sunstone.
	prolificumFlowering Plum—Prunus triloba,	Brass.
	var	Bronze.

TABLE I—(Continued)

Anther Pollen Color. Class and Sp. Hue	Specimen	CORBICULAR POLLEN
Golden yellow	Service berry—Amelanchier	Acorn (w). Raw umber (w). Golden yellow and Khaki (w).
	Altai Scotch R.—R. sp. altaica Kansas Plum—Prunus orthosepala	Khaki (w). Kangaroo.
Jonquil yellow	Roxburgh Rose—R. roxburghi	Khaki (w).
Yellow ochre	Flowering Cherry—Prunus serrulata var. Gijo	Chipmunk and Bronze.
	Fl. Cherry—Pr. ser. var. Amanogawa	Antique bronze.
REDDISH YELLOW. 585 mµ Light chrome yellow	Wreath Goldenrod—Solidago caesia	Whippet.
Lemon chrome yellow	Cornelian Cherry—Cornus mas Winter Honeysuckle—Lonicera	Yew green (w).
	fragrantissima Prairie Willow—Salix humilis Common Box—Buxus sempervir Apple—Malus sylvestris Apple—Malus sylvestris (recheck) Peach—Amygdalus persica	Yew green (w). Olive brown (w). Citrine (w). Citrine (w). Golden yellow. Olive brown and Hazel (w).
	Norway Maple—Acer platanoides Common Flowering Quince— Chaenomeles lagenaria	Acorn and Hazel (w). Lemon chrome (w).
	Bitter Wintercress—Barbarea vulgaris	Olive brown (w).
	European Euonymus—Euonymus europaeus (Spindlewood) Morrow Honeysuckle—Lonicera	Mandarinorange (w).
	morrowi Lemoine Deutzia—D. lemoinei Sapphireberry Sweetleaf—Symplocos	Khaki (w). Citrine (w).
	Pink Amur Honeysuckle—Lonicera	Raw umber (w).
	maackiYellow Sweetclover—Melilotus officinalis	Terra cotta (w). Auburn and Olive
	Roughleaf Dogwood—Cornus asperifoliaLittle Leaf Linden—Tilia cordata	Serpentine green (w). Olive brown and Citrine (w).
	White Sweetclover—Melilotus alba	Auburn and Hazel (w).
	Canada Goldenrod—Solidago canadensis Sweet Corn—Zea mays Hupeh Evodia—Evodia hupehens Frost Grape—Vitis vulpina	Whippet. Brass. Deep chrome yellow. Ivy green.
Sunflower yellow	Common Portulaca—Portulaca grandiflora. Bushy Aster—Aster dumosus. Willow—Salix sp. Glaucous Willow—Salix discolor. Peony—Paeonia albiflora & P. milkosewitchi. Fleabane—Erigeron flagellaris.	11 B 12 (Mandarin orange). Bronze yellow. Burnished gold. Yellow ochre. Tennis and Golden rod yellow. 12 J 12 (≈ Tan).

TABLE I—(Continued)

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Anther Pollen Color. Class and Sp. Hue	Specimen	CORBICULAR POLLEN
9 L 3 (≈ Sunflower y.)	Skydrop Aster—Aster patens Heath Aster—A. ericoides	Yellow ochre. Burnished gold.
Goldenrod yellow	Floating Heart—Nymphoides	Yucatan yellow.
Golden Glow (yellow)	Atlantic Goldenrod—Solidago arguta.	
	St. Bernard Lily—Anthericum liliago White Dutch Clover (recheck)	Leon yellow). Ta Ming. Antique bronze and 15 J 11 (≈ Whippet).
Chinese yellow	Japanese Pagoda Tree—Sophora japonica	Aztec.
9 E 2 (≈ Cream)	Alfalfa-Medicago sativa	Khaki.
Pinard yellow	Early Wintercress—Barbarea praecox	Olive Green and 14 L 6 (Citrine).
Popcorn yellow	Narcissus, var. Victoria—Pseudo Narcissus	Rattan.
Chrome lemon yellow	Heartleaf Aster—Aster cordifolius Flowering Crab Apple—Malus bacata.	Deep stone. 13 L 4 (≈ Bistre
	Flowering Crab Apple—Malus atrosanguinea	green). Mettalic green. Bistre green. Burnished gold.
Colonial yellow	Orpine—Sedum alboroseum	13 L 6 (Bistre gr.)
Canary yellow	Welsh Onion—Allium fistulosum Beautyberry—Calicarpa	Auburn (w). Acorn and Olive brown (w).
Ivory yellow	Eastern Redbud—Cercis canadensis Rugosa Rose—Rosa rugosa Old-fashioned Weigela—Weigla	Slate grey and Hazel (w). Slate grey (w).
	florida	Slate grey and Tan bark (w). Olive brown (w). Auburn (w). Yew green and Olive brown (w).
	Hupeh Viburnum—Vib. hupehense Viburnum—V. scabrellum Kashmir Falsespirea—Sorbaria aitchisoni Common Buglos—Anchusa officinalis.	Slate grey (w). Hazel (w). Slate grey (w). Auburn.
Yellow hue	Purple Loosesrtife—Lythrum salicaria (0.5, short anthers)	Autumn.
Chrome primrose yellow	Forsythia Japanese Maple—Acer palmatum Siberian Crabapple—Malus baccata Threelobe Spirea—Spirea trilobata	Citrine (w). Khaki (w). Olive brown (w). Olive brown (w).

COLORATION OF POLLEN

TABLE I—(Continued)

Anther Pollen Color. Class and Sp. Hue	Specimen	Corbicular Pollen
Chrome primrose yellow	Fortune Fontanesia—Fontanesia fortunei Wolf's Lilac—Syringia wolfi	Acorn (w). Olive brown and Citrine (w).
	Cockspur Hawthorn—Crataegus crusgali Japanese Honeylocust—Gleditsia japonica	Citrine (w). Citrine (w).
	American Elder—Sambucus canadensis Dusty Meadowrue—Thalictrum	Canary yellow (w).
	glaucum Ibota Privet—Ligustrum ibota	Pyrethrum yellow (w). Citrine (w).
	Narrowleaf Meadowrue—Thalictrum lucidum. Walter Dogwood—Cornus walteri Largeleaf Dogwood—C. macrophylla Chinese Chestnut—Castanea mollissima.	Olive brown (w). Citrine (w). Olive brown (w). Medium chrome
	Sword Torchlilly—Kniphofia foliosa. Wand Lythrum—Lyth. virgatum (0.5, short anthers). White Mignonette—Reseda alba Purple Loosesrife—Lythrum salicaria (0.5, long anthers).	green (w). Canary yellow (w). Yew green. Olive green. Autumn.
Chalcedony yellow	Ural Falsespirea—Sobaria sorbifolia. (Tree Falsespirea—Sorbaria arborea)	Cub (grey). Cub (grey).
Martius yellow	Honeysuckle—Viburnum sieboldi Evergreen Candytuft—Iberia sempervirens.	Olive yellow. 14 L 5 (Citrine).
9 K 1 (yellow)	·Viburnum—V. tomentosum	Canary yellow.
Chartreuse yellow	Wand Lythrum—Lyth. virgatum (0.5, long anthers)	Yew green.
Marguerite yellow	Thistle—Cirsium (afrum niveum) Climbing Hempweed—Mikania	13 G. 3 (Olive sheen)
	scandens	13 D 1 (Bronze Clair.)
9 E 1 (yellow)	Viburnum Opulus	12 K 4 (Cloudy Amber).
9 F 1 (yellow)	Jetbead—Rhodotypus scandens Rattlesnake weed—Hieracum	14 J 2 (Silver Fern).
0.7.4 (11)	venosum	Yellow ochre.
9 B 1 (yellow)	Speedwell—Veronica peduncular	Italian straw.
9 C 1 (≈ Cream)	Cohosh Bugbane—Cimicifuga racemosa Veronida Teucrium—Rupestris rosea	Beech. 12 H 3 (\approx Hay).
9 D 1 (≈ Cream)	Rhododendron	12 H 3. 13 I 3 (Olive sheen) and 15 J 4 (Olive drab).

TABLE I-(Continued)

TABLE I—(Continued)						
Anther Pollen Color. Class and Sp. Hue	Specimen	CORBICULAR POLLEN				
Greenish Yellow. 572 mµ Chrysolite green	Common Pear—Pyrus communis Pear—Pyrus Kansas Hawthorne—Crataegus coccinoides Alleghany Blackberry—Rubus allegheniensis	Egyptian Green and Marine green (w). Medium chrome green (w). Citrine (w). Slate grey and Median grey (w)				
YELLOWISH YELLOW GREEN. 568 m _{\mu} Grass green	Roughseed Clammyweed—Polanisia trachysperma	Acorn.				
Green. 535mµ Bottle green	Purple Lythrum—Lythrum salicaria	Gunmetal (Neutral grey and blue red tinge) and Deep chrome green.				
Blue Red. 448.5 m _{\mu} Egg Plant (hue)	Darwin Tulip	56 A 2+ (Kara Dagh)				
Plum (hue)	Oriental Poppy	48 E. 12 (Admiral+)				
WHITE	Persian Nepeta—Nepeta mussini Prickly Comfrey—Symphytum asperum Menzies Spirea—Sp. menziesi Common Hoarhound—Marrubium vulgare Cutleaf Chastetree—Vitex negundo. Persian Centaurea—C. dealbata Mexican Ageratum—Ageratum houstonianum. Bluestem Joepye weed—Eupatorium purpureum. Scabiosa Centaurea—C. scabiosa Brownscale Centaurea—C. jacea Peegee Panicle Hydrangea—H. paniculata grandiflora. Orange eye Butterflybush— Buddleia davidi. Panicle Hydrangea—H. paniculata Morning Glory—Convolvulvus major. White Snakeroot—Eupatorium rugosum. Caraway—Carum Carvi. Common Thyme—Thymus serpyllum vulgaris.	Dark grey (w). Slate grey (w). Slate grey (w). Smoke brown. Dark grey. Hemp. Dark grey. 13 K 5 (≈ Bistre green). 12 I 3 (≈ Hay). 11 G 3 (≈ Maple). Medium grey. Medium grey. Medium grey. Medium grey. Cub. Olive drab. Smoke Brown.				

	TAE	BLE II		
SATURATION AND	BRILLIANCY O	F THE POLLEN	LISTED	ON TABLE I

Values	In Percent	In Units	Saturation Frequency		Brilliancy Frequency	
			Anther	Corbic	Anther	Corbic
Very high plus Very high High plus High Medium plus Medium Low plus Low plus Low Very low	93.75% 87.50% 81.25% 75 % 62.50% 50 % 37.50% 25 % 12.50%	7.5 7 6.5 6 5 4 3 22 1	1 19.5 1 43 41 16.5 5	4.5 15.2 47.8 1 54 4.5	1 100.5 19.5 1 4 1 2	18.7 1 55.3 1 49 2 129

The 17 white or achromatic anther pollens are not included in the above listing.

OBSERVATIONS

Of the 146 anther pollens checked, 129 (88.3%) are colored or chromatic. 17 anther pollens were found to be white or achromatic. The samples were taken entirely at random; either as the different flowers were available in one locality or as they blossomed during the course of the two summers in 1944 and 1945. Of the colored anther pollens 57 are reddish yellow, 33 are of a yellow color and 23 are yellowish red yellow. Only 1.5 anther pollens remained unchanged in hue after being gathered by the honey-bee. One was the Common Flowering Quince, having a Lemon Chrome yellow anther pollen and it retained that color in the bees corbiculae. One-half of the bees working on the Scotch Rose, having a Golden yellow anther pollen, had corbicular pollen of that hue, the others showed Khaki colored pollen in the corbiculae. The change was due to a decrease from high saturation and brilliancy to medium saturation and brilliancy.

37.2 other anther pollens did not change in color class, but did change (increase or decrease) in saturation and nearly all of these decreased in brilliancy. All the rest, 107.3 (73%) changed in color class, in most cases also in saturation and

brilliancy.

Where two colors are reported in the corbiculae of the honey-bee working on a single species of flower, these colors were often closely related. Thus Zanzibar and Rustic brown, Slate grey and Hazel, Slate grey and Tan bark differed between themselves only in saturation. Golden yellow and Khaki, Auburn and Hazel differed only in saturation and in brilliancy, but not in color class. Other double colors differed in class and sometimes in saturation and in brilliancy.

Among the corbicular pollen colors, 28 are yellow red, 25.8 are yellowish red

yellow, 36.2 are reddish yellow, and 15 are yellow.

The anther pollen hues have a Mean wave length of $585.3 \text{ m}\mu$ and the corbicular pollen hues one of $588.8 \text{ m}\mu$. These Means were calculated from 125 variates in each series. The pollen of the Red Horse chestnut, of the Darwin Tulip, of the Oriental Poppy, change to non-spectral hues in the honey-bee's corbiculae, and one-half of the Alleghany Blackberry pollen and one-half of the Purple Lythrum pollen turn grey, while the 17 white anther pollens are achromatic. Hence these 21 variates were disregarded in the calculations. The standard deviation or amount of variability in each series is given in Table III. The coefficients of variation (Table III) show that the corbicular pollen hues are dispersed more round their Mean than are the anther pollen hues.

The difference between the Mean wave lengths is 3.5 m μ . This is significant according to Fisher's (1934) standard, D>2 x E_D , where D is the difference between the Means, and E_D is the standard error of the difference. That is it is significant if it is greater than twice its standard error. The standard error of the

TABLE III

Coefficients of Correlation Between Anther Pollen and Corbicular Pollen Hues and Other Factors, With Their Probable Error (\pm) , Their Mean Value (M), Standard Deviation (σ) , and Coefficients of Variation (c. v.).

							,
	Anther Pollen Hues	Anther Pollen Saturation	Anther Pollen Brilliancy	Corbicular Pollen Hues	Corbicular Pollen Saturation	Corbicular Pollen Brilliancy	
Anther pollen hues.		+0.4498 ±0.0481 t =6.253	0.0322 ±0.0586 t =0.359	+0.5795 ±0.0400 t =7.887	+0.1792 ± 0.0583 t = 2.020	-0.1246 ±0.0589 t =1.410	$M = 585.3 \text{ m}\mu$ $\sigma = 8.49$ c. v. = 1.45%
Anther pollen saturation.	+0.4498 ±0.0481 t =6.253		+0.0965 ±0.0588 t =1.095	+0.3170 ±0.0542 t =3.708	$+0.4080$ ± 0.0500 $t = 4.976$	-0.0697 ±0.0591 t =0.788	$M = 4.8 (60\%)$ $\sigma = 1.75$ c. v. = 36.40%
Anther pollen brilliancy.	$ \begin{array}{r} -0.0322 \\ \pm 0.0586 \\ t = 0.359 \end{array} $	+0.0965 ±0.0588 t =1.095		-0.1045 +0.0596 t =1.166	$ \begin{array}{r} +0.1118 \\ \pm 0.0591 \\ t = 1.256 \end{array} $	+0.3134 ±0.0535 t =3.719	$M = 6.7 (83.75\%)$ $\sigma = 0.76$ c. v. = 11.38%
Corbicular pollen hues.	+0.5795 ± 0.0400 t = 7.887	$ \begin{array}{r} +0.3170 \\ \pm 0.0542 \\ t = 3.708 \end{array} $	-0.1045 ±0.0596 t =1.166		+0.3610 +0.0524 t =4.604	+0.0959 +0.0597 t =1.068	$M = 588.8 \text{ m}\mu$ $\sigma = 12.37$ c. v. = 2.11%
Corbicular pollen saturation	$ \begin{array}{c} +0.1792 \\ \pm 0.0583 \\ t = 2.020 \end{array} $	$ \begin{array}{c c} +0.4080 \\ \pm 0.0500 \\ t = 4.976 \end{array} $	+0.1118 ±0.0591 t =1.256	+0.3610 ±0.0524 t =4.604		+0.5249 +0.0435 t =6.867	$M = 3.4 (42.5\%)$ $\sigma = 1.57$ c. v. = 46.29%
Corbicular pollen brilliancy.	$ \begin{array}{c} -0.1246 \\ \pm 0.0589 \\ t = 1.410 \end{array} $	$ \begin{array}{r} -0.0697 \\ \pm 0.0591 \\ t = 0.788 \end{array} $	+0.3134 +0.0535 t =3.719	+0.0959 +0.0597 t =1.068	+0.5249 +0.0435 t =6.867		$M = 3.5 (43.75\%)$ $\sigma = 1.46$ c. v. = 41.19%

difference (E_D) between the Mean pollen hues is 1.34. This was calculated from Paterson's (1939) formula: $E_D = \sqrt{E_A^2 + E_B^2}$, where E_A equals the standard error $\left(\begin{array}{c} \sigma \end{array}\right)$ of one quantity and E_B equals the standard error of the other quantity.

From the above it is evident that the difference between the Mean pollen hues is significant. Fisher's statistical "t" test gives the same result. The difference is significant if $D > t \times E_D$. According to the Table of "t," in this instance its value is 1.95 times the error of the difference 1.34 equals 2.61. The difference 3.5 in $m\mu$ exceeds it and is therefore significant.

Saturation calculations do not include the white anther pollens as they are without this attribute. 41.2 anther pollens do not decrease in saturation. 12.5 anther pollens increase their hue saturation when collected by the honey-bee. About 57% of the anther pollens decrease in saturation or become "greyer." The lower saturated anther colors show the greater change in saturation.

Mean anther pollen hue saturation is 4.8 (or 60.3%) and for corbicular pollen hues is 3.3 (or 42.4%), the difference being 1.5 (or 18.7%). This difference is greater than twice its standard error (0.20, from Paterson's formula above) and it therefore significant. Applying the "t" test gives the same result. 1.5 > 1.96 (t) x 0.20 equals 0.39.

Only 4.5 anther pollen hues do not decrease in brilliancy. The highly brilliant

hues show the greater change in brilliancy.

Mean anther pollen brilliancy, exclusive of the 17 white anther pollen samples, is 6.7 (or 83.7%) and the Mean corbicular pollen hue brilliancy is 3.5 (or 44.5%) a difference of 3.2 (or 40%). This difference greatly exceeds the value of twice its standard error, which latter is 0.14 (from Paterson's formula) and it is therefore significant. The "t" test gives the same result, and Fisher's statistical "F" and "z" tests confirm the above calculations.

Table III gives the values used in the preceding paragraphs, but not the standard error of the difference (E_D) which must be calculated separately from the formula of Paterson given above. The coefficients of variation $(c.\ v.)$ are however given. These express in percentages the dispersion of the variates around their respective means (M). The unit and decimal values for anther and corbicular pollen hue saturation and brilliancy are interchangeable with percentage values. 1 equals 12.5%, 2 equals 25%, etc. The results will be the same.

Table III also gives the coefficients of correlation (r) between anther and corbicular hues, saturation and brilliancy values. It is understood that the reaction between the given associated variables is not mutual. Anther pollen color constants are in this study the independent factors and corbicular pollen color

constants are the dependent factors.

Yule (1919) says that if the coefficient of correlation exceeds four times its probable error it is significant, otherwise its importance is primarily to show positive or negative relationship. The probable errors (following the \pm sign) given in Table III were calculated from Peter & Van Voorhis' (1940) formula:

$$PE_{r} \!\!-\!\! 0.67449 \ \frac{1 \!\!-\!\! r^{2}}{\sqrt{n}}$$

where "r" of course is the coefficient of correlation and "n" the number of variates. The more modern and critical "t" might also be used to confirm the significance of the correlation coefficients evaluated. The formula used to calculate the values of "t" are from Paterson (1939);

$$t' = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}}$$

The "t" values are given in Table III following the probable error of the coefficients of variation. From a table of "t" values it will be evident that any such value exceeding 1.959 is significant.

In every instance the anther pollen color constants show their significant influence upon the corresponding corbicular pollen color constants, the latter being the dependent factors. It will also be noticed that saturation values are closely associated with their respective hue values, but brilliancy values are correspondingly independent. Since such is the case, and the decrease in brilliancy from the anther pollen to the corbicular pollen is 40% it seems quite certain that this is the most important factor in effecting the change of color.

CONCLUSIONS

It is not by mere chance that the color changes when anther pollen is gathered by the honey-bee and packed in its corbiculae. The discussion of probable causes for this change is inferential and direct proof is not attempted.

Brilliancy is the lesser correlated factor in regard to hue and yet shows more than twice (40%) as much change as does saturation (18.7%) from the anthers to the corbiculae. Here seems to be the reason for the change of color. The decrease in brilliancy is due to a change in the surface texture of the pollen mass when it is removed from the anthers and formed into pellets in the corbiculae of the honeybee. Pollen grains are quite freely and loosley attached to the anthers and present en massé, a sufficiently rough surface to cause a diffuse reflection of the incident

light and consequently affect to a lesser degree the wave lengths which produce the color sensations. When these same pollen grains are gathered by the honey-bee they are compressed by the auricle on the anterior end of the planta of the hind leg of the bee and thus squeezed upward into the pollen basket or corbicula to form the pollen pellet or corbicular pollen mass. In the process of packing the bee also uses the middle legs to pat down the pellets thus formed and render them more compact. This relatively smoother mass surface reduces the diffuse reflection of light and hence alters to a degree in spectral composition the light waves that produce the color sensations. This may be illustrated by comparing the pollen grains to freshly fallen snow. Pollen grains are to a great extent as transparent as ice-crystals and otherwise vie in beauty by their sculpturing with the myriad forms of snowflakes. Freshly fallen snow is a diffuse reflector of light, but when it is trodden down or a crust forms over it this property of reflection is reduced. In like manner when pollen is packed by the bee in the corbiculae it looses much of its brilliancy.

Another reason for the change of color is the addition of moisture to the pollen when it is taken from the anthers of the flowers and packed in the corbiculae. Parker (1922) notes this factor when he says that pollen of two different colors was collected from bees working on the Acer negundo, "but upon drying these (i. e., pollen masses) only one color was persistent." In the pollen packing process very little pollen reaches the corbiculae without becoming thoroughly moist and sometimes the pollen masses are fairly liquid with moisture. This addition of moisture, mostly honey, dulls still further the natural brilliancy of the anther pollen, hence the corbicular pollen masses often appear as if "water-logged." According to Todd and Bretherick's (1942) analysis, corbicular pollen contains a mean 11.6% of water while hand collected, i. e., anther pollen has a mean 9.72% water content. Vivino and Palmer (1944) give a mean 23.89% moisture value for corbicular pollen after partial drying for 24 hours. Todd and Bretherick (1942) also note the presence of water-soluble (and fat-soluble) types of pigments in pollen. Any addition therefore of moisture to anther pollen during the transfer to the corbiculae would naturally tend to dilute the pigment and change the color. Chemical analysis indicates that honey is predominantly used for this moistening function and hence honey solids and water content increase disproportionately in corbicular pollen.

Phillips (1929) showed that even water-white honey absorbs some of the light waves, in the following amounts: blue light, 36%, yellow-green light, 23% and red light 18.5%. Even though the honey-bee uses a comparatively small amount of honey in the pollen packing process this fluid coating on the pollen grains absorbs some of the light waves, twice as much blue as red, and one and one-half as much yellow-green as red. This might account for a slight shift of the mean anther hue of the pollen from the 585.3 m μ toward the reddish end of the color spectrum, or toward the mean corbicular hue of 588.8 m μ .

Since the ether extract in pollen may also contain pigments, its decrease would effect a change of color. Todd and Bretherick (1942) give evidence of this decrease. Hand collected, i. e., anther pollen contains a mean 5.01% ether extract

while corbicular pollen contains a mean 4.96% ether extract.

Another factor may be the addition of foreign matter to the corbicular pollen masses in the process of packing by the not always fastidious honey-bee. Corbicular pollen masses under the microscope show bits of fiber, dust, gritty matter resembling tiny particles of coal-like mineral matter, that do not stain and are impervious to the point of a probing needle. Some of these minutiae were estimated at from 200 or more microns in length or breath. Pollen grains range in size from 4.5 microns, the smallest (Forget-me-not) to 200 microns in diameter for the largest (Pumkin). In the corbicular pollen of the White Sweet Clover distinctive lighter colored "flakes" appeared but the effect in this case was to

"lighten" the pollen pellets. No count was made of this foreign matter nor was it identified, but its presence generally tends to darken the corbicular pollen masses. While anther pollen is also exposed to contamination from dust in the air, soil, etc., the probability for greater contamination is increased for the honey-bee due to its greater range, its flight activity from flower to flower, and its hairy body serving as a "catch all" for such foreign particles.

SUMMARY

Pollen literature ignores to a great extent the important factor of color. literature seldom is a distinction made between anther and corbicular pollen when

speaking of the color of pollen.

Introspectively defined colors may be translated into mathematical terms, by the use of color constants and approximate wave lengths may be assigned to practically all hues. Using a standard color chart, anther and corbicular pollen

may be identified by color-matching.

Two seasons' field work show for the 146 specimens examined about ten general classes of color, as yellowish red, red yellow, yellow, greenish yellow, etc., and about one hundred distinct hues as, Cherry red, Coral 1ed, Henna, Rustic brown, etc. Most anther pollens (88.3%) are colored chromatically, the rest are white. Only 1.5 pollens were observed to remain unchanged in color when packed by the honey-bee in its corbiculae. Changes in color may be due to decrease in saturation or a decrease in brilliancy or both, and the changes are significant.

Saturation is closely correlated with its associated hue but brilliancy enjoys greater freedom. Corbicular pollen color constants are the dependent factors in relation to anther pollen color constants, but preserve among themselves the same

relationship as do the anther pollen color constants.

Brilliancy's decrease is the important factor in the change of color between anther and corbicular pollen. The decrease is caused by a change of surface texture in the pollen masses, the addition of moisture, absorption of light waves by the honey used in packing the pollen, dilution of water-soluble pigments and the addition of foreign matter. Hence corbicular pollen differs in color from its corresponding anther pollen.

LITERATURE CITED

Alfonsus, Erwin C. 1933. Some Sources of Propolis. Gleanings Bee Culture. 61 (2): 92.

American Jt. Committee on Horticultural Nomenclature. 1923. Prepared by F. L. Olmstead, F. V. Coville, and H. P. Kelsey. 564 pp. Salem, Mass.

Armbruster, L., and J. Jacobs. 1934-5. Pollenformen und Honigherkunft-Bestimmung. Bucher des Archiv fur Bienenkunde. 2: 1-122.

Armbruster, L., and G. Oenike. 1929. Die Pollenformen also Mittel zur Honig-Herkunftsbestimmung. Bucherei fur Bienenkunde. 10: 1-116.

Bisson, Charles S., and George H. Vansell. 1940. Investigations on the Physical and Chemical Properties of Beeswax. U. S. Dept. Agr. Tech. Bul. 716.

Casteel, D. B. 1912. The Behavior of the Honey Bee in Pollen Collecting. U. S. Dept. Agr. Bur. Ento. Bul. 121.

Cheshire, F. R. 1888. Bees and Beekeeping; scientific and practical. 2 Vol. London.

Erdtman, G., and H. Erdtman. 1933. The improvement of pollen analysis technique. Svensk

Erdtman, G., and H. Erdtman. 1933. The improvement of pollen analysis technique. Svensk Bot. Tid., 27 (3): 347–357.

Fischer, Hugo. 1890. Beitrage zur vergelichenden Morphologie der Pollenkorner. 72 pp.

Berlin.

Fisher, R. A. 1934. Statistical Methods for Research Workers. 319 pp. Edinburgh.
Judd, D. B. 1940. Hue Saturation and Surface Colors. Jour. Opt. Soc. Amer. (and Rev. Sci. Instruments). 30: 2-

Mace, Herbert. 1944. The Beekeeping Annual. Harlow, Essex. 28 pp.
Maerz, A., and Rea, Paul M. 1930. A Dictionary of Color. 208 pp. New York.
Massachusetts Institute of Technology. 1936. Handbook of Colorimetry.
Nutting, P. G. 1913. Bulletin Bureau Standards. 9; No. 187.
Parker, R. L. 2922. Some Pollens Gathered by Bees. Report of State (Iowa) Apiarist for 1922. 68-75.

Paterson, D. D. 1939. Statistical Technique in Agricultural Research. 263 pp. New York.

- Peters Charles C., and Van Voorhis, Walter R. 1940. Statistical Procedures and their Mathematical Bases. New York.

- Peters Charles C., and Van Voorhis, Walter R. 1940. Statistical Procedures and their Mathematical Bases. New York.
 Phillips, E. F. 1929. Uses and cause of colors in honey. Gleanings in Bee Culture. 57: 288.
 Rood, Ogden. (1942?). Funk & Wagnalls Standard Dictionary. "Spectrum."
 Sempers, J. F. 1911. The Colors of Pollen. Gleanings in Bee Culture. 39: 341.
 1912. A Stampede for the Pollen of Wild Carrots. Gleanings on Bee Culture. 40: 384.
 Sladin, F. W. L. 1912. Further Notes on How the Corbicula or Pollen Basket is loaded with Pollen. Gleanings Bee Culture. 40: 700.
 Todd, Frank E. 1941. Pollen and Nectar Studies. Gleanings Bee Culture. 69: 14.
 Todd, Frank E., and Ormond Bretherick. 1942. The Composition of Pollens. Journal Econom. Entomology 35 (3): 312-317.
- Entomology 35 (3): 312-317.

 Vansell, Geo. H. 1942. Factors Affecting the Usefulness of the Honeybees in Pollination.
 U. S. Dept. Agr. Cir. 650.

 Vivino, A. Earl, and L. S. Palmer. 1944. The Chemical Composition and Nutritional Value of Pollens Collected by Bees. Arch. Biochemistry 4: 2, 129-136.
- Webster's New International Dictionary of the English Language. 1942 G. & C. Merriam Co., Springfield.
- Wodehouse, R. P. 1935. Pollen Grains, Their Structure, Identification and Significance in
- Science and Medicine. 574 pp. New York.

 Young, W. J. 1908. A Microscopic Study of Honey Pollen. U. S. Dept. Agr. Bur. Chemistry Bul. 110: 70–88.
- Yule, G. Udny. 1919. An introduction to the theory of statistics. 398 pp. London.