Genetics of Flower Color in *Asystasia gangetica*, Linn.[1]

H. KAMEMOTO and W. B. STOREY[2]

ASYSTASIA GANGETICA, LINN., also known synonymously as *Justicia gangetica*, Linn. and *A. coromandeliana*, Nees. (Alston, 1931) is a procumbent, clambering herb of the plant family Acanthaceae. It is commonly called "zigzag plant" from its habit of growth. It is indigenous to the tropics of the Old World from Malaya to Africa.

The time of its introduction into the Hawaiian Islands is unknown. From a few scattered plants of 20 years ago, it has multiplied rapidly and become widely distributed through the island group. It flowers and sets seeds profusely under a wide range of environmental conditions, from wet to dry and from sunny to shady. It grows luxuriantly in partial shade, which has led to its adoption as an ornamental plant for growing around buildings and along fences. It is propagated vegetatively very easily by means of cuttings.

Purple appears to predominate among the flower colors of wild plants of *A. gangetica* in the Hawaiian Islands, but white and yellow are also to be seen. Plants with pale purple, white, yellow, and pink flowers are recorded in the literature (Hooker, 1885; Bailey and Bailey, 1946; Bailey, 1942; Neal, 1948). The occurrence of a number of variants in color of flower, and the ease of propagation and culture make the species a favorable subject for a genetical study of flower color.

A. gangetica has leaves that are opposite, exstipulate, and ovate to cordate with the margins entire. The inflorescence is a terminal raceme 5–12 cm. in length, ultimately bearing 10 to 15 flowers. The flower is gamopetalous, with an inflated tube and an expanded limb. The tube is straight, and about 2.5 cm. in length. The limb comprises five lobes, and measures about 3 cm. across. There are four epipetalous stamens which are inserted in pairs on the tube about halfway between the base and the throat. The ovary is superior. It develops into a 2-celled, 4-seeded capsule about 2.5 cm. in length, which when mature dehisces with explosive force, usually around midday. The seeds are dark, thick-coated, flattened, and more or less circular in outline measuring about 4 mm. in diameter.

Excepting in those flowers which have purple coloration on the outer epidermis of the limb extending into the dorsal portion of the corolla tube, the tube is pale yellow. Even the white flower which has a white limb has a pale yellow tube. In flowers that have the inner epidermis of the corolla limb colored purple, the median lower lobe is darker than the rest of the limb.

MATERIALS AND METHODS

Several different flower color types were gathered from a number of localities in the vicinity of the city of Honolulu. These were grown in the greenhouse and used as parents. Descriptions of the flowers of the parental plants follow:

1. Yellow with inner epidermis of limb

[1] Published with the approval of the Director of the University of Hawaii Agricultural Experiment Station as Technical Paper No. 301. Manuscript received December 16, 1953.

[2] Assistant Horticulturist and Horticulturist, University of Hawaii Agricultural Experiment Station.

medium purple, resulting in a bronzy color. (From Pensacola Branch Station of the Hawaii Agricultural Experiment Station.)

2 and 16. Inner epidermis of limb dark purple and outer epidermis of limb and back of tube purple. (From University Avenue roadside.)

3, 4 and 5. Entire limb yellow. (From Pensacola Branch Station.)

6, 14 and 15. Yellow with inner epidermis of limb pale purple resulting in a light bronzy color. (From Pensacola Branch Station.)

7. Inner epidermis of limb white and outer epidermis of limb and back of tube purple, resulting in light pinkish color. (From Army Port and Service Command Nursery.)

8. Inner epidermis of limb yellow and outer epidermis and back of tube purple resulting in a light bronzy color. (From Army Port and Service Command Nursery.)

9. Entire limb white. (From Army Port and Service Command Nursery.)

10 and 13. Inner epidermis of limb dark purple, and outer epidermis of limb and back of tube purple. (From Army Port and Service Command Nursery.)

11. Inner epidermis of limb dark purple and outer epidermis of limb and back of tube pale purple. (From Army Port and Service Command Nursery.)

12. Inner epidermis of limb dark purple. (From Army Port and Service Command Nursery.)

17. Entire limb white. (From University Avenue roadside.)

18. Entire limb white. (From Pensacola Branch Station.)

Culture

All plants were grown in the greenhouse. When the seeds from the first crosses were planted, they germinated sporadically. The germination period extended over several months, and it required about a year before the complete recording of the flower types could be made. This difficulty was overcome when Akamine (1947) found that the seeds undergo a period of dormancy, and that by treating them in petri dishes with alternating temperatures, dormancy may be broken, resulting in their germination within a period of two weeks. The germinated seeds were then transplanted into soil. By employing Akamine's technique, the recording of flower types was greatly facilitated, for the plants came into flowering early and at approximately the same time.

It was also found that *A. gangetica* responds to day length; that is, it makes primarily vegetative growth during the summer months while it flowers profusely during the rest of the year. It takes about six months for the plants to flower if the seeds are planted in early summer. On the other hand, during the winter months it takes only three to four months from the time of planting of seed to the first flower.

Pollination

The flower of *A. gangetica* is protandrous. On the day of anthesis the pollen is shed, usually completely by noon. However, the stigma is not receptive until the following day when the upright style bends at the stigmatic end and a sticky substance is exuded on the stigmatic surface. The period of receptivity of the stigma is two to three days. For emasculation the corolla of a newly opened flower is pulled off in the morning carrying the stamens with it and leaving the pistil exposed. Pollination is done the following morning when the pollen from a newly opened flower of a desired type is applied by touching an anther to the sticky stigmatic surface. It was necessary to cross a number of flowers to get a large population, for from one pollinated flower the maximum number of seeds obtainable is four. Actually, an average of less than three seeds per capsule was obtained.

The parental plants were selfed to see whether they were true breeding. Upon segregation of the offspring the ratios were recorded. Several crosses among the parental plants were made, and the resulting F_1 types noted. The F_1 plants were subsequently selfed or, where convenient, backcrossed to get the segregating ratios.

RESULTS AND DISCUSSION

Flavone Colors

WHITE. With the white flowers, the limb of the corolla is self-white, but the tube is pale yellow, as mentioned earlier. The whites were found to breed true for white. Also, when two whites were crossed, only whites were obtained. When these white flowers were exposed to ammonia vapor, the corolla turned yellow as it does in many other flowers (Buxton, 1932; Lawrence, 1931; Mehlquist, 1939; Scott-Moncrief, 1936). When such a reaction takes place, it is believed that an ivory anthoxanthin, probably apigenin, is present, which upon treatment with ammonia is converted into a yellow anthoxanthin, probably luteolin.

YELLOW. The yellow pigment in this species is a water soluble flavone, which turns orange when placed in ammonia vapor, similar to that found in Dahlias (Lawrence, 1931). The coloration occurs in the inner epidermal layer of the limb as well as the hypodermal layers. The yellow parent plants bred true for yellow. When yellow flowers were crossed with white flowers, all of the F_1 flowers were yellow. The subsequent F_2 generation segregated into a 3:1 ratio and the backcross to the white into a 1:1 ratio. The results are shown in Table 1.

Yellow, then, is a simple dominant to white and *Y* has been designated as the gene necessary for the production of yellow pigment. Gene *Y* when homozygous recessive results in white flowers in the absence of other flower colors.

Anthocyanin Colors

A close examination of the purple colored flowers revealed that the anthocyanin sap pigment occurs in two definite patterns, one in which the pigment is found in the inner epidermal layer of the limb and the other in which the coloration occurs in the outer epidermal layer of the limb and extends to the base of the tube. In order to make a distinction between the two patterns of coloration, the former will be designated hereafter as purple, and the latter as purpleback, due to the fact that the back of the tube is colored in addition to the outer epidermis of the limb. The anthocyanin pigment is basically purple, with its variations ranging from bluish purple to purplish red. It appears that where the background is white, bluish purple seems to exist, whereas when the background is yellow, the color tends to be reddish purple or bronze. The purple pigment also seems to be affected by the light intensity, and the age of the blossom. With aging, the color tends to turn bluish.

TABLE 1
THE RATIO OF YELLOW TO WHITE FLOWERS IN THE F_2 AND BACKCROSS PROGENIES OF *White* × *Yellow* IN *Asystasia gangetica*

PROGENY	OBSERVED		CALCULATED		CHI-SQUARE
	Yellow	White	Yellow	White	
F_2	33	9	31.5	10.5	.285
Backcross	25	26	25.5	25.5	.020

Chi-square = 3.841 at 5% level.

TABLE 2
THE RATIO OF PURPLE TO NON-PURPLE FLOWERS IN THE F_2 GENERATION OF CROSSES BETWEEN PURPLE AND NON-PURPLE IN *Asystasia gangetica*

PROGENY	OBSERVED		CALCULATED		CHI-SQUARE
	Purple	Non-Purple	Purple	Non-Purple	
(12 × White) Selfed......	69	16	63.75	21.25	1.729
(11 × White) Selfed......	74	27	75.75	25.25	.162
(1 × White) Selfed......	9	3	9	3	.000
(6 × White) Selfed*.....	84	22	79.5	26.5	1.019
Total..................	236	68	228.0	76.0	1.123

Chi-square = 3.841 at 5% level.
* The cross *6 x White* resulted in both purple and non-purple. The F_2 here is the result of selfing the purple form.

PURPLE. The parental plants 1, 11, 12, and 16 were determined to be homozygous for purple. Parental plant 6 was heterozygous for purple, segregating into 3 purple to 1 non-purple. When flowers homozygous for purple were crossed to white, the resulting F_1 progenies were all purple. In the F_2 generation as shown in Table 2 the combined ratios were 236 purple to 68 non-purple, a good 3:1 ratio, indicating that purple is dominant to non-purple. The symbol *P* has been assigned to the gene responsible for the production of purple color. Gene *P* when homozygous recessive will result in white flowers in the absence of other flower colors.

It was observed in the first few crosses between dark purple and non-purple forms that the flower color of the F_1 generation was slightly less intense than the purple parent and that colors of the F_2 generation segregated into light and dark forms. This suggested the possibility of additive effects of gene *P*. Upon selfing pale purple parent 6, which was heterozygous for that factor, the offspring segregated into 3 purple to 1 non-purple, and among the purple progeny, both pale and dark forms appeared in the ratio of 21 pale to 7 dark. Selfing a dark form resulted in all dark purple offspring, revealing the homozygous condition of that dark colored first selfed generation plant. Selfing a pale form, on the other hand, resulted in a segregating progeny of 3 purple to 1 non-purple again, which establishes the heterozygous nature of the pale purple first selfed generation plant. The ratio of light and dark forms appearing among the purple forms was 31 to 11 which is not a significant deviation from a 2:1 ratio.

In another case, a dark purple plant, Number 12, was crossed to white, and resulted in light purple F_1 plants. The F_2 generation segregated into 3 purple to 1 white and among the purple forms 49 were light purple and 20 dark purple. This ratio fits a 2:1. Thus, it appears that the gene for purple is cumulative in its effect, that *PP* produces dark forms, *Pp*, light, and *pp*, whites. Such an additive effect of gene for the production of flower color is not new in plants. In *Nicotiana* hybrids, Smith (1937) demonstrated that the heterozygous condition has a diluting effect. Also in Dahlias (Lawrence, 1931) the two genes, *A* and *B*, that determine anthocyanin color are cumulative in their effect.

In addition to the cumulative action of gene *P* a modifying gene or genes may be operating to intensify or dilute the anthocyanin pigment, for not all flowers homozygous for *P* have the same intensity of pigment and not all segregating progenies can be subjected to simple genetic analysis. Further studies are necessary to clarify the inheritance of various intensities in coloration.

When purple flowers are crossed to yellow flowers, the purple on yellow background results in a purplish red, or bronzy color which is easily distinguishable from purple appearing on a white background. For convenience, the interaction of purple and yellow will be referred to as bronze, and purple on white background as purple. The cross between purple and yellow resulted in all bronze flowers. The F_2 generation segregated out into 9 bronze, 3 purple, 3 yellow, and 1 white, indicating that yellow and purple are independently inherited (Table 3).

PURPLEBACK. It was mentioned earlier that the anthocyanin pigment which occurs in the outer epidermal layer of the limb extends to the back of the tube. It was found that parents 2 and 16 bred true for purpleback. Parent 8, having a yellow limb with purpleback, parent 7, having white limb with purpleback, and parent 11, having purple limb with purpleback, were found to be heterozygous for this character.

Parent 16, which is homozygous for purpleback, when crossed with yellow, gave all purpleback offspring. The F_2 generation of this cross together with a few others are given in Table 4. The combined ratios of purpleback to non-purpleback was 166:56, which fits a 3:1 ratio. Purpleback, *B*, exhibits a simple dominance to non-purpleback, *b*.

Tables 5 and 6 show the dihybrid ratios between the factors *P* and *B* and *Y* and *B* respectively. In either case, the ratios obtained fit 9:3:3:1, indicating that *B* is independently inherited from genes *P* and *Y*.

Basic flower color in *A. gangetica* is governed by three sets of genes: *P*, producing

TABLE 3

THE RATIO OF BRONZE (*PY*), PURPLE (*Py*), YELLOW (*pY*), AND WHITE (*py*) FLOWERS RESULTING FROM FAMILIES HETEROZYGOUS FOR *P* AND *Y* IN *Asystasia gangetica*

PROGENY	OBSERVED				CALCULATED				CHI-SQUARE
	Bronze (PY)	Purple (Py)	Yellow (pY)	White (py)	Bronze (PY)	Purple (Py)	Yellow (pY)	White (py)	
(6 × White) Selfed	66	18	16	6	59.625	19.875	19.875	6.625	1.674
(12 × Yellow) Selfed	89	32	25	12	88.875	29.625	29.625	9.875	1.369
Total	155	50	41	18	148.5	49.5	49.5	16.5	2.491

Chi-square = 7.815 at 5% level.

TABLE 4

THE SEGREGATING RATIO OF PURPLEBACK TO NON-PURPLEBACK FROM FAMILIES HETEROZYGOUS FOR PURPLEBACK IN *Asystasia gangetica*

PROGENY	OBSERVED		CALCULATED		CHI-SQUARE
	Purple-back	Non-Purple-back	Purple-back	Non-Purple-back	
(16 × 5) Selfed	70	24	70.5	23.5	.109
(7 × 14) Selfed	31	8	29.25	9.75	.419
(11 × White) Selfed	17	7	18	6	.223
(7 × 3) Selfed	48	17	48.75	16.25	.046
Total	166	56	166.5	55.5	.007

Chi-square = 3.841 at 5% level.

TABLE 5

SEGREGATING RATIOS OF *PB* (PURPLE WITH PURPLEBACK), *Pb* (PURPLE WITH NON-PURPLEBACK), *pB* (NON-PURPLE WITH PURPLEBACK), AND *pb* (NON-PURPLE WITH NON-PURPLEBACK FROM FAMILIES HETEROZYGOUS FOR *P* AND *B* IN *Asystasia gangetica*

PROGENY	OBSERVED				CALCULATED				CHI-SQUARE
	PB	Pb	pB	pb	PB	Pb	pB	pb	
(11 × White) Selfed	11	6	6	1	13.5	4.5	4.5	1.5	1.630
(7 × 14) Selfed...	42	9	18	2	40.41	13.56	13.56	4.49	4.490
Total..............	53	15	24	3	53.46	17.82	17.82	5.94	4.048

Chi-square = 7.815 at 5% level.

TABLE 6

SEGREGATING RATIOS OF *YB* (YELLOW WITH PURPLEBACK), *Yb* (YELLOW WITH NON-PURPLEBACK), *yB* (WHITE WITH PURPLEBACK), AND *yb* (WHITE WITH NON-PURPLEBACK), FROM FAMILIES HETEROZYGOUS FOR *Y* AND *B* IN *Asystasia gangetica*

PROGENY	OBSERVED				CALCULATED				CHI-SQUARE
	YB	Yb	yB	yb	YB	Yb	yB	yb	
(7 × 14) Selfed....	25	6	6	2	21.96	7.32	7.32	2.44	.972
(7 × 3) Selfed....	33	13	15	4	36.54	12.18	12.18	4.06	1.041
Total..............	58	19	21	6	58.5	19.5	19.5	6.5	.170

Chi-square = 7.815 at 5% level.

purple pigment in the inner epidermis of limb, *B*, producing purple in the outer epidermis of limb and back of tube, and *Y*, producing yellow in the entire limb. Each of these is inherited in a simple Mendelian fashion and independently from one another. White flowers result only when all three are present as homozygous recessives. The interaction of the three genes with one another gives rise to the following eight phenotypes, when dark purple (*PP*) and pale purple (*Pp*) are classed together.

P- Y- B- bronze with purpleback
P- Y- bb bronze with non-purpleback
P- yy B- purple with purpleback
P- yy bb purple with non-purpleback
pp Y- B- yellow with purpleback
pp Y- bb yellow with non-purpleback
pp yy B- white with purpleback
pp yy bb white with non-purpleback

A plant heterozygous for *P*, *Y*, and *B* can be expected to give rise to a progeny of all eight classes in the typical trihybrid ratio of 27:9:9:3:9:3:3:1. It can be seen that from such a progeny, the chance occurrence of a white form is only one out of 64 plants. This explains the rather limited number of white forms appearing among open-pollinated seedlings in nature. Also, if the bronze forms are included with purples, the preponderance of purple-colored types is to be expected on the basis of inheritance of flower color in this species.

The genotypes of the parental plants collected for this study have been determined as follows:

Plant No.	
1	*PP YY bb*
2, 16	*PP yy BB*
3, 4, 5	*pp YY bb*

6, 14, 15	*Pp YY bb*
7	*pp yy Bb*
8	*pp Yy Bb*
9, 17, 18	*pp yy bb*
10, 13	*PP yy Bb*
11	*PP yy Bb*
12	*PP yy bb*

These plants represented all phenotypes except one, bronze with purpleback, *P Y B.*

SUMMARY AND CONCLUSIONS

The mode of inheritance of flower color in *Asystasia gangetica* Linn. was determined. Yellow flower color is dependent on gene *Y* which is dominant to white.

Two patterns of anthocyanin pigmentation exist, one in which the inner epidermis of the limb is colored, and the other in which the outer epidermis of the limb and the back of the tube are colored. The gene *P* produces the former pattern while the gene *B* is responsible for the latter. Gene *P* was determined to be additive in its effect; *PP* results in dark forms while *Pp* in light forms.

Genes *P*, *Y*, and *B* are inherited independently of one another. The dihybrid F_2 segregants fit the 9:3:3:1 ratios if the pale and dark purple forms are classed together. Where purple anthocyanin occurs together with yellow pigment, a bronzy color results. With the three genes for flower colors operating, eight basic phenotypes are obtainable. Since white flower color is due to the recessive condition of all three genes, *pp*, *yy*, *bb*, the limited number of such forms appearing in open pollinated progenies is explained.

REFERENCES

AKAMINE, E. K. 1947. Germination of *Asystasia gangetica* L. seed with special reference to the effect of age on the temperature requirements for germination. *Plant Physiol.* 22: 603–607.

ALSTON, A. H. G. 1932. In Trimen, H. *Handbook to the flora of Ceylon.* Vol. 6, 350 pp. Dulau & Co., London.

BAILEY, L. H. 1942. *Standard cyclopedia of horticulture.* Vol. 1, 1200 pp. The MacMillan Co., New York.

BAILEY, L. H. and E. Z. BAILEY. 1946. *Hortus Second.* 778 pp. The MacMillan Co., New York.

BUXTON, B. H. 1932. Genetics of the primrose, *P. acaulis. Jour. Genet.* 25: 195–205.

HOOKER, J. D. 1885. *Flora of British India.* Vol. 4, 780 pp. L. Reeves & Co., London. 4: 387.

LAWRENCE, W. J. C. 1931. The genetics and cytology of *Dahlia variabilis. Jour. Genet.* 24: 257–304.

MEHLQUIST, G. A. L. 1939. Inheritance in the carnation, *Dianthus caryophyllus*, I. Inheritance of flower color. *Amer. Soc. Hort. Sci., Proc.* 37: 1019–1021.

NEAL, M. C. 1948. *In gardens of Hawaii.* 805 pp. Bernice P. Bishop Mus. Honolulu.

SCOTT-MONCRIEF, R. 1936. A biochemical survey of some mendelian factors for flower colors. *Jour. Genet.* 32: 117–170.

SMITH, H. H. 1937. Inheritance of corolla color in the cross *N. langsdorfii* by *N. Sanderae. Genetics* 22: 347–360.