INFLUENCE OF GENES CONTROLLING FLOWER COLOR ON RELATIVE QUANTITIES OF ANTHOCYANINS AND FLAVONOLS IN PETALS OF IMPATIENS BALSAMINA[1]

CHARLES W. HAGEN, JR.

Botany Department, Indiana University, Bloomington, Indiana

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IN earlier studies concerned with the genetic regulation of pigmentation in the garden balsam, *Impatiens balsamina*, the genes which determine the nature of anthocyanin pigmentation were found in individual cases to regulate also the structure of leucoanthocyanins (ALSTON and HAGEN 1958) and flavonols (CLEVENGER 1958). Thus the dominant gene *H*, while increasing the quantity of pelargonidin derivatives present in the flowers, also allows production of a leucopelargonidin, which does not occur when the recessive allele, *h*, is homozygous. At another locus, *L* governs production of anthocyanins derived from malvidin and at the same time introduces a polyhydroxy flavonol, apparently myricetin. The simplest interpretation of these observations would indicate that an intermediate made available for anthocyanin synthesis may also be exploited for leucoanthocyanin and flavonol synthesis and, therefore, that both *L* and *H* operate at an early stage in the elaboration of the flavonoid structure.

Additional control of flower color in the balsams is provided by a series of alleles *p*, P^g and P^r. While slight anthocyanin pigmentation may occur with *p*, the principal influence of these genes seems to be an augmentation of anthocyanin production, *p* being the least effective of the series and P^r the most effective. This effect is clear when the quantities of anthocyanin pigments produced in each genotype are determined, and the relation of the alleles at the *p* locus to flavonol synthesis can be assessed by taking advantage of certain features of the ultraviolet absorption by this group of compounds.

METHODS

The four lateral petals from each of two freshly opened flowers on the same plant were collected and immediately extracted with small (2–3 ml) portions of hot 95 percent ethanol containing 0.4 percent HCl (w/v). The extraction was repeated 5–6 times or until the yield was negligible and the petal residue was essentially colorless. The extracts were pooled and made to either 10 or 25 ml with the extracting solvent in volumetric flasks, the degree of dilution depending on the intensity of pigmentation of the original flowers. A further dilution was frequently necessary to bring absorbance within the range of the spectrophotometer.

Absorption spectra in the visible range were obtained with the Bausch and

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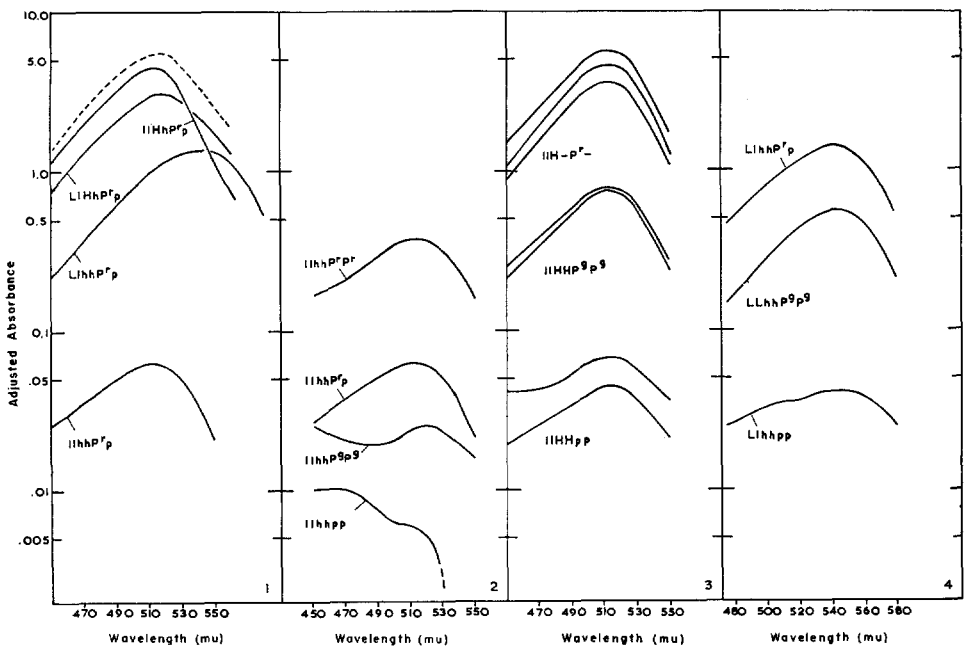
Lomb "Spectronic 20". Ultraviolet spectra were measured with the Beckman Model DU spectrophometer. Where chelation of flavonols was desired, one ml of two percent aluminum chloride prepared in the extraction solvent was added to three ml of extract. On the assumption of a linear relation between absorbance and dilution, each absorbance value has been adjusted to represent the absorbance of an extract of the four lateral petals from one flower in ten ml of extract.

RESULTS

Figure 1 illustrates the interaction of dominant genes in producing the magenta phenotype. The four plants providing these extracts were segregants of a backcross between an inbred white-flowered plant and a heterozygous magenta-flowered plant (*llhhpp* × *LlHhP*^{r}*p*). Consequently all the plants were heterozygous for the genes indicated. *P*^{r}, when other genes are recessive, produces a moderate pigmentation of the petals with pelargonidin derivatives. With *H* present also, the pelargonidin content is 50-fold greater, thus illustrating the synergistic action of these two genes. When *L* is present with *P*^{r}, λ max shifts in correspondence with the replacement of pelargonidin by malvidin, but the total absorbance is almost as great as with red flowers (*llHhP*^{r}*p*). Magenta flowers (*LlHhP*^{r}*p*) exhibit the



FIGURES 1–4.—Absorption in visible wave lengths by extracts of petals of *Impatiens balsamina*. FIGURE 1. Interaction of genes in forming the magenta phenotype. FIGURE 2. Action of genes at the *p* locus in the absence of other dominant pigmentation genes. FIGURE 3. Interaction of alleles at the *p* locus with *H*. FIGURE 4. Interaction of alleles at the *p* locus with *L*. For further explanation see text.

intermediate spectrum expected with a mixture of pelargonidin and malvidin derivatives. If this single example is representative, the total anthocyanin production is somewhat less than the sum of production in *LlhhP^{r}p* and *llHhP^{r}p* as presented in the dotted curve of Figure 1. The shape and position of the magenta curve indicate that the deficiency in total pigment is largely at the expense of pelargonidin rather than malvidin. Other instances in which the synthesis of malvidin seems to have a competitive advantage over the synthesis of pelargonidin have been noted in *Impatiens balsamina* (ALSTON and HAGEN 1958).

Figure 2 illustrates pigment production under the influence of alleles at the *p* locus in the absence of other dominant genes. The lowermost curve is derived from an extract of a white flower and serves to illustrate the low background provided by components under the control of factors other than the genes considered here. The minor absorption at the blue end of the spectrum may account for irregularities in such low curves as the one directly above (*llhhP^{g}P^g*).

The same figure illustrates one of the few effects of heterozygosity which have yet been observed in extracts. The curve designated *llhhP^{r}p* is the higher of two analyses of flowers of the same heterozygous plant, the lower yielding an adjusted absorbance of 0.036 at 512 mu. Three separate homozygous plants of two different cultures and genetic origins gave maximal absorbances between 0.23 and 0.37. The effect of gene dosage can also be observed grossly in faintly colored genotypes involving *L* (*Llhhpp vs. LLhhpp* or *LlhhP^{g}P^g vs. LLhhP^{g}P^g*) and less certainly in those involving *H*, but it cannot be detected in intensely colored flowers.

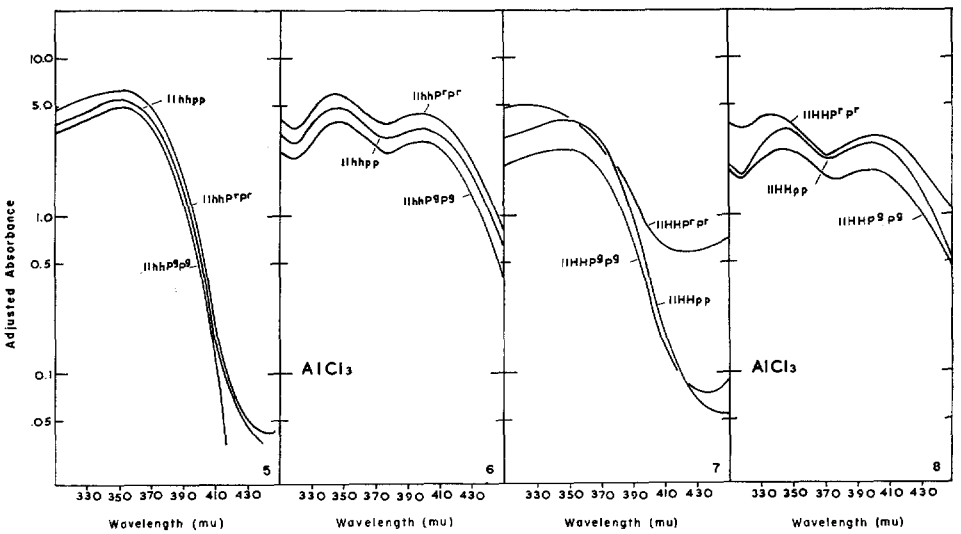
The failure of gene dosage to influence pigmentation of intensely colored flowers is illustrated by the three upper curves of Figure 3. The central curve in this group is derived from a plant of the genotype *llHhP^{r}p*, the uppermost was *llHHP^{r}P^r* and the lowermost was either *llHHP^{r}P^r* or *llHHP^{r}P^g*. Nine analyses of plants including *llHHP^{r}P^r*, *llHHP^{r}P^g*, and *llHhP^{r}p* yielded absorbances at λmax between 2.55 and 5.50, but there was no evident relation between the genotype and the maximal absorption.

The remaining curves of Figure 3 demonstrate the influence of alleles at the *p* locus in regulating total anthocyanin production. *P^g* produces a ten-fold increase in absorbance over *p*, and *P^r* produces at least a five-fold increase over *P^g*. Approximately the same relation is observed in the curves for malvidin derivatives presented as Figure 4.

With the exception of the genotype *llHHP^{r}P^r*, absorption of wave lengths between 300 and 450 mu was remarkably uniform. Each curve exhibited a broad maximum extending from 340 mu to 360 mu (Figures 5 and 7). Flowers with the genotype *llHHP^{r}P^r* consistently yielded greater extinctions at 330 mu and at wave lengths longer than 390 mu.

After addition of aluminum chloride, two peaks appeared with maxima at 345 mu and near 400 mu (Figures 6 and 8). Again the genotype *llHHP^{r}P^r* was an exception with the peak at shorter wave lengths lying at 335 mu.

Absorbances in the ultraviolet range were somewhat greater in extracts of plants recessive for *h* than in those carrying the dominant allele but the differ-



FIGURES 5–8.—Absorption by extracts of petals of *Impatiens balsamina* in ultraviolet wave lengths with and without aluminum chelation. FIGURE 5. Nonchelated extracts from genotypes differing in alleles at the *p* locus. Other pigmentation genes are recessive. FIGURE 6. The same extracts which appear in Figure 5 after adjustment to 0.5 percent aluminum chloride. FIGURE 7. Nonchelated extracts from genotypes carrying *H* but differing in alleles at the *p* locus. FIGURE 8. The same extracts which appear in Figure 7 after adjustment to 0.5 percent aluminum chloride. For further explanation see text.

TABLE 1

Absorbances in ultraviolet wave lengths by chelated and nonchelated extracts of flowers of Impatiens balsamina. *Values are adjusted to the equivalent of four petals from one flower in ten ml of solvent.*

	Absorbance in 95 percent ethanol containing 0.4 percent HCl w/v			Absorbance in 95 percent ethanol containing 0.4 percent HCl w/v and 0.5 percent $AlCl_3$ w/v				
Genotype	No.	Range, 345 mu	Mean, 345 mu	No.	Range, 345 mu	Mean, 345 mu	Range, 400 mu	Mean, 400 mu
lhp	4	4.19–5.47	5.00	3	4.24–4.95	4.68	3.48–3.56	3.51
lhP^g	5	3.66–7.19	5.23	1	4.15	4.15	3.00	3.00
lhP^r	6	4.42–6.38	5.17	2	4.60–6.00	5.30	3.30–4.38	3.84
lHp	4	3.10–4.81	4.11	3	2.83–4.00	3.46	2.13–3.28	2.77
lHP^g	4	2.21–4.91	3.60	2	2.25–2.55	2.40	1.70–1.90	1.80
lHP^r	5	3.35–4.50	3.72	2	2.55–4.18	3.36	1.85–3.20	2.52
–h–	15	3.66–7.19	5.14	6	4.24–6.00	4.80	3.00–4.38	3.54
–H–	13	2.21–4.91	3.80	7	2.25–4.18	3.67	1.70–3.28	2.69

ences were not correlated with the quantities of anthocyanins produced (Table 1).

The flowers providing chelated extracts were collected during December and January. Plants grown at later seasons yielded similar curves from alcoholic extracts but a higher proportion of the absorbing materials was unaffected by aluminum chloride, the long wave peak showing as an inflection point. The

seasonal effect has not been analyzed beyond noting that a period of three weeks on short-day (9 hour) regimen begun after initiation of buds failed to produce "winter-type" absorption in summer grown flowers. Interpretation of this phenomenon is not essential for the purposes of this paper and the absorption by chelated extracts of summer grown plants has been excluded from Table 1.

DISCUSSION

The spectra presented in Figures 1 through 4 represent summed absorption by several glycosides of each anthocyanidin present (ALSTON and HAGEN 1958) and each of these glycosides may have somewhat different spectral characteristics. Consequently no attempt is made to compute pigment content. The curves serve to illustrate the magnitude of influence of each gene and the interaction of genes at different loci. Absorption by other components is apparently negligible in this range.

The genes H and P^r have approximately equal effect on pelargonidin production. When present together each supplements the action of the other for the total pigmentation is much greater than the sum of pigmentation produced by separate action. In a similar fashion P^r potentiates the action of L in the formation of malvidin derivatives. It seems apparent, therefore, that the p locus controls a general reaction in anthocyanin biosynthesis which is independent of the hydroxylation pattern exhibited by the ultimate pigment.

The similarity in structure between anthocyanins and other flavonoid compounds has suggested a common synthetic pathway in early stages (ROBINSON 1936). This assumption is supported by evidence of competition for common precursors between different classes of flavonoids as first noted in dahlia by LAWRENCE and SCOTT-MONCRIEFF (1935) and is also suggested by cases in which a single gene governs substitution in two related nuclear structures. Examples of the latter exist in *Impatiens balsamina* where L controls production of the flavonol myricetin and the anthocyanin malvidin and H regulates the quantity of pelargonidin derivatives as well as introducing a leucopelargonidin. Competition between flavonols and anthocyanins has not been noted on chromatograms prepared from extracts of balsams.

All genotypes of *Impatiens balsamina* which lack the gene L yield petal extracts in which free kaempferol and a single kaempferol glycoside are the only conspicuous flavonols (CLEVENGER 1958). Kaempferol exhibits maximal absorption at 266 mu and 367.5 mu (JURD and GEISSMAN 1956) and its glycosides should absorb at somewhat shorter wave lengths. All flavonoid pigments as well as many simpler phenolic compounds absorb strongly at wave lengths between 250 and 300 mu. As a consequence, extracts of balsam flowers exhibit great optical density in this region. At longer ultraviolet wave lengths, however, flavanones, catechins and leucoanthocyanins contribute little to the total extinction. Among the common flower constituents which have absorption maxima in this region are flavones and flavonols, cinnamic acid derivatives and chlorogenic acid. While these components of balsam petals have not been completely analyzed, it is apparent that

chlorogenic acid is not a significant component. Moreover the cinnamic acids absorb at somewhat shorter wave lengths than the flavones and flavonols (JURD 1956).

Further characterization of the pigments responsible for ultraviolet absorption can be achieved by examining absorption by the metal chelates. JURD and GEISSMAN (1956) have demonstrated that aluminum chelation between the 3-hydroxyl and 4-carbonyl groups produces a large bathochromic shift of the long-wave ultraviolet maximum, in the case of kaempferol from 367.5 mu to 426 mu. Chelation occurs readily at this position in neutral or mildly acid solutions whereas chelation at other susceptible positions, such as 4-carbonyl, 5-hydroxyl or 3', 4'-dihydroxyl, causes large shifts of absorption maxima only in basic conditions. The major absorption by apigenin, which lacks the 3-hydroxyl group, occurs in ethanol at 326 mu; in ethanol with added aluminum chloride at 342.5 mu.

Glycosidic combination at the 3-position of flavonols might be expected to reduce the bathochromic shift by blocking chelation involving the 3-hydroxyl group. GAGE and WENDER (1949), however, show marked shifts after aluminum chelation of rutin (quercetin-3-rutinoside), xanthorhamnetin (quercetin-3-trirhamnoside) and robinin (kaempferol-3-robinoside-7-rhamnoside). Such combination may provide other chelation sites.

Since kaempferol and its glycoside are prominent constituents of balsam petals and these compounds are susceptible to aluminum chelation, changes in kaempferol concentration should be observable through direct spectrophotometric examination of balsam petal extracts. The curves obtained are precisely those anticipated where absorption by kaempferol and its glycoside is supplemented by the absorption of another component(s) at slightly shorter wave lengths. The other component is apparently incapable of chelation under the conditions of these measurements.

The observations show no action of genes at the *p* locus on flavonol production. Where anthocyanin production can be altered 50-fold by genetic means, absorption due to flavonols remains essentially constant. Because absorption by components which show lesser shift after addition of aluminum also remains constant, other prominent absorbers in this region must also be unaffected by alleles at the *p* locus.

Flavonol production is somewhat less with the gene *H* than in the presence of its recessive allele, but the difference is independent of alleles present at the other loci and also independent of the total quantity of anthocyanin produced. If this gene diverts some intermediate from flavonol synthesis, the intermediate must be partitioned between anthocyanins and other still undetected substances which exist in competitive equilibrium with pelargonidin derivatives.

The shape of the ultraviolet absorption curve is somewhat different in the genotype *llHHP*^{r}*P*^{r} than in other genotypes examined. As this is also the genotype which produces the greatest amount of pelargonidin, it is possible that the greater absorption at 330 mu and at wave lengths above 390 mu is caused by derivatives

of pelargonidin itself which has minor absorption peaks at these wave lengths (KARRER 1932).

There is no evidence in *Impatiens balsamina* for competition between anthocyanins and flavonols of the sort existing between anthocyanins and yellow pigments in dahlias (LAWRENCE and SCOTT-MONCRIEFF 1935) or between anthocyanins and aurones in snapdragons (JORGENSEN and GEISSMAN 1955). Moreover the genes which augment anthocyanin production do not provide intermediates which are employed directly for increased flavonol synthesis. Although this information is essential for interpretation of the action of genes which regulate pigmentation in this species, there are still many alternative operations which could be governed by genes at the *p* locus.

SUMMARY

In *Impatiens balsamina*, the allelic genes *p*, P^g and P^r control the quantity of anthocyanins in petals without affecting flavonol production. The gene *H* effects a reduction in kaempferol content which is independent of the amount of pelargonidin produced.

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