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PATTERN PARTITIONING OF FLORAL FLAVONOIDS IN THE *MIMULUS LUTEUS* COMPLEX

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Key Word Index—*Mimulus luteus* complex; Scrophulariaceae; herbacetin; herbacitrin; floral patterns; flavonoid patterning; floral flavonoid regulation; coordinate control.

Abstract—Some flavonoids are differentially distributed between the spots and the background in the flowers of the *Mimulus luteus* complex; others are not. Two pigments that consistently co-occur with the anthocyanin in the floral spots are the flavone and flavonol with the same B-ring hydroxylation pattern as the anthocyanin. The flavonol and anthocyanin are also similarly sugar substituted. It is suggested that patterning depends, in part, on coordinate control of members of a parallel biosynthetic series. One pigment that is exclusive to background tissue is the visible yellow flavonol, herbacetin 7-glucoside. Carotenoids are not pattern partitioned. It is concluded that the roles of the two yellow pigments differ.

INTRODUCTION

THE MAJOR task of flavonoid distribution studies has been to identify those pigments present in entire organs. Little is written concerning the distribution of flavonoids among the variously patterned sections of a single flower. Harborne[1] asserts that patterns are usually the result of a localized pigment increase or, alternatively, the superimposition of an additional pigment on those generally present. Pattern partitioning of floral flavonoids in the *Mimulus luteus* L. complex, however, reveals a different situation—one that is particularly interesting from the point of view of flavonoid pattern control.

RESULTS

Selected red spots of fresh flowers were excised and their pigments extracted. Approximately an equal amount of background tissue was similarly treated. Two dimensional paper chromatograms were examined for pigments present. The results are summarized in Table 1.

DISCUSSION

Some pigments vary in their partition between patterned and acyanic areas. Apigenin 7-glucoside, for example, may occur in spots only, in background only, in both, or in neither. Quercetin 7-glucoside and kaempferol 7-glucoside may occur throughout the petal, although the latter is sometimes pattern partitioned. Without exception, quercetin 3-glucoside is produced where cyanidin 3-glucoside is produced, whether pattern contained or diffuse. Both pigments are glucosylated at the 3-position and both are 3′, 4′-dihydroxylated.

Synthetic ability for specifically substituted flavonoids engenders the production of a parallel pigment series.[2] Within certain tissues in this complex, the two B-ring series found are those with 4′- and 3′,4′-hydroxyl groupings. The pigment-pattern correlation data suggest that control of the members of a series may be coordinate. Luteolin 7-glucoside,

[1] J. B. HARBORNE, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), p. 247, Academic Press, New York (1965).

[2] J. B. HARBORNE, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 593, Pergamon Press, Oxford (1962).

the flavone with the same B-ring hydroxylation pattern as cyanidin and quercetin (flavone structure precludes *O*-glycosylation at the 3-position), displays the same tendency to be present in spots and absent in background tissue, although there are flowers in which it is not produced at all. This absence may reflect its lesser biosynthetic kinship; it is still suggested that luteolin 7-glucoside *control* is coordinate with the other members of the series, for this pigment is never produced in acyanic areas.

TABLE 1. *Mimulus luteus* COMPLEX PIGMENT PRODUCTION; PATTERN VS. BACKGROUND

Population	Area	A7G	Q3G	K3G	L7G	Q7G	H7G	K7G	C3G	Car
tig 5016	L	+	+	tr	+	+	−	+	+	−
	P	+	+	tr	+	+	−	+	+	−
	B	tr	−	−	−	tr	−	+	−	−
tig 5016	L	+	+	−	+	+	−	+	+	
× *lut* 5042	B	+	−	−	−	+	+	+	−	
tig 5016	L	+	+	−	+	−	−	−	+	tr
× *tig* 5303	B	+	−	tr	−	−	−	+	−	−
lut 5042	L	+	+	+	+	+	−	−	+	
× *tig* 5303	B	+	−	−		+	+	+	−	
lut 6161	L	+	+	+	+	+	−	+	+	
× *lut* 5042	B	+	−	−	−	+	−	+	−	
lut 6650	L	−	+	tr	−	+	−	+	+	+
× *lut* 5042	B	+	−	−	−	+	+	+	−	+
lut 6680	L	+		−		+	−	+	+	+
	B	−		−		+	+	+	−	+
lut 6680	L	+	+	+	+	+	−	−	+	+
× *tig* 5303	B	tr	−	−	−	+	+	+	−	+
lut 6685	L	−	+	−	−	+	−	+	+	+
× *tig* 5303;F_2	B	−	−	−	−	+	+	+	−	+
lut 7517	L	+	+	−	+	+	−	+	+	+
× *lut* 5042	B	+	−		−	+	−	−	−	+
lut 7518	L	+	+	−	−	+	−	+	+	
	B	−	−	−	−	+	−	+	−	
lut 7518	L	+	+		+	+	−	+	+	+
× *lut* 5042	B	−	−		−	+	+	+	−	+
lut 7518	L	+	+	+	+	+	−	+	+	+
× *tig* 5303	B	+	tr	tr	−	+	+	+	−	+
lut 7531	L	+	+		−	−	−	tr	+	+
	B	+	−	−	−	−	−	tr	−	+
lut 9565	L	+	+				−	+	+	tr
× *lut* 5042	B	+	−	−	−	+	+	+	−	tr
	D	+	+	+	+	+	−	+	+	tr

Key. lut: *M. luteus*, *tig*: *M. tigrinus*, B: background tissue, D: area of diffuse anthocyanin production, L: labellum spot, P: petal spots, A7G: apigenin 7-glucoside, Q3G: quercetin 3-glucoside, K3G: kaempferol 3-glucoside, L7G: luteolin 7-glucoside, Q7G: quercetin 7-glucoside, H7G: herbacetin 7-glucoside, K7G: kaempferol 7-glucoside, C3G: cyanidin 3-glucoside, Car: carotenoids.

Herbacitrin (8-hydroxykaempferol 7-glucoside) and the anthocyanin are in all cases mutually exclusive. The one population (*lut* 7517 × *lut* 5042) that did not produce kaempferol 7-glucoside in background floral tissue contained no herbacitrin either. A dependence of herbacitrin on the presence of kaempferol 7-glucoside would not be surprising and suggests that the former arises by hydroxylation of the latter; however, the hybrid may merely lack the requisite genotype.

An intense yellow color characterizes herbacitrin. The only other visible flavonoid is the anthocyanin. The juxtaposition of these two pigments enhances visible pattern contrast.

Carotenoids modify color throughout the flower, shifting spots towards the red (from the magenta color of cyanidin) while intensifying background yellow.

Yellow flavonols are rare. Their occurrence in widely scattered plant groups deprives them of broad taxonomic significance.[3] The carotenoids are the more usual source of yellow flower color. Yellow flavonoids, in general, usually co-occur with carotenoids; in some plants yellow flavonoids have specifically displaced them.[3,4] The presence of herbacitrin together with carotenoids seems redundant. When *Mimulus* patterning is considered, however, it does not. It is obvious that patterning arose in *Mimulus* by localization of flavonoid production; carotenoids are uniformly present. It is therefore suggested that the evolution of yellow flavonoids and their displacement of carotenoids in disjunct plant groups is a reflection of their ease of insertion into an already existing regulatory system, coupled with their intensification of pattern effects in the visible range.

Floral patterns in *Mimulus* are the result of a well developed control system that establishes singular flavonoid complexes in specific floral areas. Other plants should be examined to establish whether the findings discussed herein more generally pertain.

EXPERIMENTAL

Plant material. The *Mimulus luteus* complex comprises four species (*M. cupreus*, *M. laceratus*, *M. luteus*, and *M. tigrinus*). Plants of the complex were grown from seed in the University of Utah greenhouse. Only fresh flowers were used.

Pigment extraction. Excised floral tissue was covered with cold 0·01 N methanolic HCl and soaked for 48 hr at 4°. The tissue was removed and the extract was concentrated to dryness at room temp. The wall of the evaporating flask was washed down with a few drops of methanol. The extract was used directly for chromatography.

Chromatography. Two dimensional chromatography was accomplished by standard techniques[4] on Whatman No. 1 paper, using BAW 4:1:5 and 15% HOAc.

Identification. Pigments were identified by their appearance and position on PCs, as previously established.[5,6]

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[3] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[4] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[5] A. M. FERRO, M. S. Thesis, University of Utah (1971).
[6] G. J. BALDWIN, M. S. Thesis, University of Utah (1972).