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Flavonoid Genetics of the 28-chromosome "Siberian" *Iris*

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Summary. A chromatographic survey of flavonoids in the various flower color mutants of the 28-chromosome "Siberian" *Iris* (series *Sibiricae* subseries *Sibiricae*) was conducted using mutants of known genotype (Vaughn 1974). Mutants at the C locus contain the malvidin glycoside ensatin, indicating that this gene locus may control methylation of delphinidin. Clear white, a mutation at the W locus, results in the production of flavones in excess.

Key words: Siberian *Iris* - Flavonoid Genetics - Anthocyanin - Flavone

Introduction

Although Werkmeister (1960, 1969) reported the occurrence of the anthocyanin malvidin in the 40-chromosome "Siberian" species *Iris chrysographes* Dykes, little or no data on the flavonoids of the cultivars of the 28-chromosome Siberian *Iris* (series *Sibiricae* subseries *Sibiricae*, Lenz 1976) has been accumulated. Only five major classes of blossom color are known in this group: blue-purple, red, pink, dull white, and clear white. The pigment genetics of this group has been reviewed by Tiffney (1971) and Vaughn (1974), showing that only two loci (C and W) are involved in the control of anthocyanin production in these color types. Thus, it may be possible to establish the functions of these two loci in the flavonoid pathway by examination of flavonoids from mutants of known genotype. This paper summarizes the flavonoid data obtained from these mutants.

Materials and Methods

Plant Material

Iris plants were obtained from a variety of sources, which include the author's own breeding program. Color and genotype of varieties are summarized in Table 1.

Extraction and Chromatography

Pigments were extracted from whole fresh blossoms in equal volumes of 2N HCl with mortar and pestle and stored in the cold (4-9°C) until used. Pigment extracts were spotted approximately 1/2" from the base of strips or sheets of Kodak MN cellulose (both with and without fluorescent indicator) with extra fine capillary pipets. Chromatograms were run by ascent in one dimension in either BAW (4 parts 1-butanol : 1 part glacial acetic acid : 5 parts H_2O, upper phase, v/v, prepared within three hours of its use) or 15% HAc in H_2O (by volume) and in two dimensions in the same two solvents. Chromatographic chambers were allowed to saturate before chromatograms were run.

Resultant chromatograms were first viewed in visible light and long wave U.V. and then fumed over NH_3 vapor and viewed in both visible light and long wave U.V. again.

Reference compounds were obtained from the following *Iris* cultivars: 'Floridor', which contains floridorin, tulipanin, mangiferin, and irigenin (Werkmeister et al. 1966); a Japanese *Iris* cultivar which contains ensatin (Takeda and Hayashi 1964); 'Blue Ribbon', which contains delphanin, swertisin, and several other flavones (Asen et al. 1970). Each was co-chromatographed with the Siberian *Iris* pigment extracts for comparison.

Results and Discussion

The distribution of anthocyanins as found in the 28-chromosome Siberian *Iris* is summarized in Table 1. At least 10 chromatograms were run of each genotype and results were consistent in all cases.

It is interesting to note that all of the mutations at the C locus (Vaughn 1974) have the malvidin glycoside ensatin, which may indicate that the C locus is responsible for the methylation of delphinidin to malvidin (Werkmeister 1960). Although phenotypically the c^r gene behaves as a Mendelian recessive (Tiffney 1971; Vaughn 1974), in the heterozygous condi-

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Table 1

Cultivar	Class	Genotype	Anthocyanin (s)
'Vaughn G-3'	blue-purple	C C WW	delphanin[1]
'Towanda Redflare'	red	$c^r c^r$ WW	delphanin, ensatin[2]
'Mildred Peck'	pink	$c^p c^p$ WW	ensatin
'Caesar's Ghost'	dull white	c c WW	ensatin
'Snow Flare'	clean white	C C ww	no anthocyanin[3]
'Vaughn TP-1'	blue-purple	C c^r WW	delphanin, ensatin

[1] delphanin = 3-p-coumaryl-rutinoside, 5-clucoside of delphinidin
[2] ensatin = 3-p-coumaryl-rutinoside, 5-glucoside of malvidin
[3] swertisin, a C-glycosyl flavone (Asen et al. 1970) found as an intense spot

tion C c^r, both delphanin and ensatin are produced (Table 1). Thus the gene may be more correctly written C^R to indicate codominance, at least at the molecular level, with C. Tiffney (1971) has reported that individuals of C c^p genotype are more blue-lavender than C C individuals, inferring a presence of ensatin, and indicating that c^p may also behave as a codominant. The C c combination has not been sufficiently tested to comment on at this time.

In comparison with other types of *Iris*, the 28-chromosome Siberians have a very small number of other flavonoids present (Vaughn 1976). Although swertisin (Asen et al. 1970) was found in 'Vaughn G-3' and 'Snow Flare' and a luteolin glycoside in both 'Mildred Peck' and 'Caesar's Ghost', no other co-pigment molecules were found on the chromatograms (Vaughn 1976).

Homozygous w w individuals seem to have a block in the anthocyanin pathway so that flavones are produced in excess (Vaughn 1976). Oddly, the same C-glycosyl flavone (swertisin) produced in the bulbous *Iris tingitania* Boiss. and Reut. cv. 'Blue Ribbon' (Asen et al. 1970) is found in these rhizomatous Siberian *Iris* mutants.

The recent decision of Lenz (1976) to retain the 28- and 40-chromosome Siberian *Iris* in a single series is further substantiated by the finding of a malvidin glycoside in a "red" variety of each group (Werkmeister 1960, 1969). A thorough review of the flavonoids of the 40-chromosome Siberian *Iris* should further aid in substantiating or negating Lenz's arrangement.

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