PRECURSORS AND GENETIC CONTROL OF PIGMENTATION

3. DETECTION AND DISTRIBUTION OF DIFFERENT WHITE GENOTYPES OF BLUEBELLS (*ENDYMION* SPECIES)

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SUMMARY

Three white forms of *Endymion non-scriptus* (L.) Garcke were identified which: (a) synthesised cyanidin from dihydroquercetin (DHQ); (b) remained acyanic when treated with DHQ; (c) contained colourless anthocyanin pseudobase and were thus considered to be very pale pink forms. The frequency of these types in central Norfolk was determined. Type (b) constituted 10^{-3} of the population in one area; the other two were fairly evenly spread and formed only about 10^{-6} of the population. The anthocyanin pigments of this species and of *E. hispanicus* were analysed.

1. INTRODUCTION

ANTHOCYANIN synthesis has been induced in suitable acyanic genotypes of certain flowers, notably *Antirrhinum majus*, by feeding precursors in the biosynthetic pathway (Stickland and Harrison, 1974) and this enabled a rapid analysis of some hypostatic genes involved in pigment synthesis often within 24 hours (Harrison and Stickland, 1974). During a general survey of acyanic flowers two types of white *Endymion non-scriptus* (L.) Garcke were found, only one of which produced anthocyanin from the precursor dihydroquercetin (DHQ). The nature of the natural and induced anthocyanins and the distribution of the various genotypes are reported.

2. METHODS

Materials and methods were as reported by Stickland and Harrison (1974). Anthocyanin analysis followed the procedures of Harborne (1967). The presence of any anthocyanin pseudobase was demonstrated by immersion of a perianth segment in 0.1 per cent HCl in methanol at room temperature (or cooler); a pink-purple colouration appeared within about 1 minute.

Frequencies were based on the number of flower spikes; pink- and white-flowered plants were counted individually, but the overall numbers in the population were estimated from a count of 20 random square metres and the total area occupied. The area surveyed is shown in fig. 1. The grid is the 1 km squares of the British National Grid and the letters at the 00/00 intersection identify the adjacent 100 km squares.

3. RESULTS

Endymion non-scriptus

This species occurs widely in woods where the soil is not too wet in winter nor too dry in summer. A total of 8×10^7 flower spikes was observed.

The predominant darkly pigmented blue form (at least 99.9 per cent) contained delphinidin-3-(*p*-coumarylglucoside)-5-glucoside (table 1) as reported by Harborne (1967). Forms with pale blue flowers occasionally contained the corresponding cyanidin glycoside. The comparatively rare pink-flowered form also contained this cyanidin glycoside, but it was usually

FIG. 1.—Area surveyed for *Endymion* species. The map is divided into 1 km squares; ordinates are National Grid references (see Methods). Shaded areas are towns: 1, East Dereham; 2, Watton; 3, Hingham; 4, Wymondham. ●, woods in which white or pink forms were found amongst the blue; ○, small woods of that type. ■, woods in which only blue forms were found; □, small woods of that type. In both, the area of the solid spot is proportional to estimates of the total number of flowers (1 mm² : 10⁶ flowers approx.). X, woods in which no *Endymion* flowers were found.

TABLE 1
Natural and induced anthocyanins in Endymion species

Species	Petal colour	Pg-3-CG-5-G	Cy-3-G	Cy-3, 5-G ₂	Cy-3-CG-5-G	Dp-3-G	Dp-3-CG-5-G
<i>Non-scriptus</i>	White		I	I	I		
	(W(+))form						
	Pink	t	I	I	+	tI ^a	
	Blue		I	tI	t ^b	tI ^a	+
<i>Hispanicus</i>	White		I	I	I	tI ^a	
	(W(+))form						
	Magenta			tI	+		
	Blue		tI	tI	+		+

+, present; t, trace present; I, induced by feeding DHQ; tI, trace induced; Pg, pelargonidin; Cy, cyanidin; Dp, delphinidin; C, *p*-coumaryl; G, glucoside.

^a Trace of Dp-3-G observed in only one flower of each type.

^b Pale blue flowers occasionally contained small amounts of this compound.

accompanied by a little of the corresponding pelargonidin glycoside, not previously noted in this species. In these blue- and pink-flowered forms, the bract under each flower was tinged with the same colour as the flower. Two specimens (growing 30 cm apart) had anthocyanin confined to discrete blue spots (plate I, A), possibly due to an unstable gene similar to the *pallida-recurrens* allele in *Antirrhinum majus*. Another comparable sample of a flaking flower was found near Lyme Regis by Dr R. W. Riding (personal communication).

When dihydroquercetin (DHQ) was administered to these blue and pink flowers, cyanidin-3-glucoside and its 3,5-diglucoside appeared (table 1), mainly on a line up the centre of each perianth segment. In one specimen of each colour, production of a trace of delphinidin-3-glucoside was noted.

White flowered specimens were of three kinds. One form contained anthocyanin as pseudobase: these flowers appeared white when fully open, but the buds usually had pink tips to the perianth segments. The bracts often (16/49 spikes) had a purplish colour, in contrast to the translucent pale green of the normal white flowered specimens. This form was thus considered to be a very pale pink and was included with the other pink specimens. The other two forms always had white flowers with no pseudobase; one, $W(+)$, produced anthocyanin when treated with DHQ and the other, designated $W(-)$, was unresponsive. The anthocyanins formed by $W(+)$ were the 3-glucoside, the 3,5-diglucoside and its *p*-coumaryl derivative, of cyanidin.

Feeding of dihydrokaempferol (DHK) to pink and $W(+)$ flowers led to the formation of the same cyanidin glucosides as were synthesised from DHQ. Even in flowers naturally producing a little pelargonidin, no increased pelargonidin production was found after DHK treatment. Naringenin induced the same result as DHQ in many pink flowers, but anthocyanin production was always much less than from DHQ. Naringenin was not effective in $W(+)$ flowers, and thus these presumably had a genetic block between flavanone and flavanonol (Harrison and Stickland, 1974).

There was considerable variation in the amount of anthocyanin in the flowers designated pink. Only four specimens were seen in which mature flowers were definitely pink and even in these the pigment was distributed unevenly. However, if the pseudobase-containing flowers were included, these forms represented about 10^{-6} of the total population and were fairly evenly distributed (fig. 2). One specimen was found in a small wood (TG136077) and this gave an anomalous high frequency of about 2×10^{-5} .

$W(+)$ forms were even rarer, with a frequency of about 10^{-7} (fig. 3). In contrast, the frequency of $W(-)$ forms was as high as 10^{-3} in the woods near TG078056 (fig. 4). This frequency fell with distance away from this point until, at the periphery of the area examined, frequencies between 10^{-6} and 10^{-7} were found.

The rare $W(+)$ forms were found only in woods that contained both pink and $W(-)$ types. Pinks however occurred in one wood where there were no $W(-)$, and $W(-)$ in 10 woods where there were no pinks. However in the woods near TG078056, where there were large numbers of $W(-)$ flowers, it would only be expected (from the frequencies elsewhere) that about 10^{-3} of the white population would be either $W(+)$ or pink (possibly very pale pink). Although one pale pink was detected, it is very likely that other $W(+)$ or pinks remained undetected.

FIG. 2.—Frequency of pink flowers. Key in fig. 3.

FIG. 3.—Frequency of $W(+)$ flowers.

FIG. 4.—Frequency of $W(-)$ flowers. Key in fig. 3.

Endymion hispanicus

This species is not native to England and is usually only found in gardens. Most of the specimens examined came from the vicinity of Hingham (3 in fig. 1).

The main anthocyanins were the same as in *E. non-scriptus*. In blue flowers, delphinidin-3-(*p*-coumarylglucoside)-5-glucoside was always accompanied by the corresponding cyanidin compound (table 1). Magenta flowers (darker than the pink *E. non-scriptus*) contained this cyanidin glycoside, but no pelargonidin. Imbibition of DHQ yielded essentially the same pigments as in *E. non-scriptus* (see plate I, B).

In this species there was a clear distinction between magenta and white forms; no pseudobase-containing white forms were found. The majority of the white flowers produced cyanidin glycosides when fed DHQ, that is, were $W(+)$. One produced a trace of delphinidin-3-glucoside. A few specimens were found in which there was no induced anthocyanin formation, that is $W(-)$.

Since these plants were deliberately introduced, no attempt was made to record the frequencies of the various colour forms, which were often specifically selected. In many instances the three colours were of approximately equal frequency.

4. DISCUSSION

The presence of anthocyanin pseudobases in plants has not often been noted. Harborne (1967, p. 242) reported their presence in *Iris* flowers, while Reznik and Neuhäusel (1959) recorded them in the submerged green

parts of water plants. Besides *Endymion non-scriptus*, the white flowers of several species, such as *Malcomia maritima*, *Malva moschata*, *Matthiola incana*, *Primula obconica*, and *Silene dioica* have also been seen to contain pseudobase. Often these white flowers are tinged pink or purple at the bud and/or wilting stages. All flowers containing pseudobase have produced anthocyanin when treated with precursors, and most notably with DHQ, to which they respond quickly.

The distribution of the pink and white, $W(+)$, forms of *E. non-scriptus* was fairly even and the frequency can reasonably be accounted for by chance mutation. However the $W(-)$ forms had a 1000-fold higher frequency in one locality. The main wood (Forehoe Big Wood, ref. TG078056) is well established and has been in existence for at least 180 years. There is no evidence of the introduction of *E. hispanicus* which might have influenced the proportion of white, $W(-)$, flowers (although local forms of white *E. hispanicus* are at present predominantly $W(+)$). This wood lies at the northern extremity of Kimberley Park. The Earls of Kimberley could have introduced unusual strains of white flowered forms in Forehoe Big Wood. Other woods nearer to Kimberley Hall (grid. ref. TG091047) might have been expected to be so treated first, and since the proportion of $W(-)$ forms in these is 100-fold lower, this explanation appears less likely. There are few other published measurements of the frequencies of white-flowered *Endymion* plants. Wilson (1959) gave the numbers of white spikes in certain woods, for two of which it would appear the frequency was about 10^{-6} of the population. From the data of R. W. Riding (1977 and personal communication), frequencies of 10^{-3} - 10^{-4} were found in two woods near Widnes and one in the Isle of Man; a hillside at Ilfracombe had a density of about 10^{-6} - 10^{-7} , whereas woods near Lyme Regis were probably of both categories. In none of these cases was distinction made between $W(+)$, $W(-)$ and possible pseudobase-containing very pale pinks.

In south-west Essex, one wood near Harlow contained $W(-)$ plants at a frequency of 5×10^{-6} (F. G. W. Stickland, personal communication). Out of 8×10^6 *Endymion* spikes observed these were the only non-blue forms seen, apart from a few pink and $W(+)$ types which there was reason to believe were hybrids with *E. hispanicus*. Near Penzance in Cornwall, since *Endymion* also grows along the hedgerows, there are no distinct communities and it was difficult to calculate frequencies. Over the total area surveyed (a 5 km radius from Penzance containing 1.2×10^6 flower spikes), pink flowered specimens constituted about 10^{-5} of the population and $W(-)$ forms about 4×10^{-6} (L. H. Stickland, personal communication). In restricted localities the frequency of both types could be 10^{-4} or even higher.

From these figures it would appear that the white-flowered plants do occur at a relatively high frequency in other parts of the country.

It is unlikely that the higher frequency of the $W(-)$ form arises from the progression: Wild type (blue) $\rightarrow W(+)$ (frequency $\approx 10^{-7}$) $\rightarrow W(-)$ (10^{-3}). The sequence: Wild type $\rightarrow W(-) \rightarrow W(+)$ is numerically possible but again unlikely since $W(-)$ is a late block in the biosynthetic pathway analogous to the *pallida* type of *Antirrhinum majus* (Stickland and Harrison, 1974) and $W(+)$ is an intermediate block (*incolorata* type): thus to obtain $W(+)$ from $W(-)$ implies mutational change involving both blocks which, if similar to the controls in *A. majus*, could be regulated by two unlinked genes. Although the $W(+)$ mutants could theoretically arise from $W(-)$,

PLATE I, A (top).—*Endymion non-scriptus* with blue pigment in discrete spots compared with the unstable *pallida-recurrens* allele in *Antirrhinum majus*.

PLATE I, B (bottom).—*Endymion hispanicus* (magenta): normal (left) and after treatment with dihydroquercetin (right).

it does appear on genetical grounds to be unlikely. It is therefore suggested that some *E. non-scripta* populations preponderantly produce one of the two pure white mutant forms. In some populations both mutant types occur and it is probable that these are of independent origin.

Riding (1977 and personal communication) has examined the frequency of whites in other areas and recorded the flower, capsule and seed number and seed viability of both blue and white forms. Unfortunately, it was not then known that the white flowers could be classified into *W*(-) and *W*(+) forms. Although there was a reduction in seed formation, but not in seed viability, in the white forms, it is difficult to decide the significance of these results in connection with the production of white-flowered plants since the seed from the whites mainly produced blue-flowered plants. This indicates the high degree of outcrossing in *E. non-scripta* and the origin and maintenance of white forms within a population remains conjectural.

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