GEISSMAN, T. A., AND LISCHNER, H. 1952. Flavanones and related compounds. VII. The formation of 4, 6, 3', 4'-tetrahydroxy-2-benzylcoumaranone-3 by the sodium hydrosulphite reduction of quercetin. *J. Am. Chem. Soc.*, *74*, 3001-3004.

GILBERT, R. I. 1971. An unusual anthocyanin in *Antirrhinum majus*. *Phytochem.*, *10*, 2848-2849.

GILBERT, R. I. 1972. Pelargonidin-3-glucoside in *Antirrhinum majus*. *Phytochem.*, *11*, 2360.

GILBERT, R. I. 1973. Chalcone glycosides of *Antirrhinum majus*. *Phytochem.*, *12*, 809-810.

GRISEBACH, H. 1972. Enzymology and regulation of flavonoid biosynthesis in plants and plant cell cultures. *Phytochem.*, *11*, 862-863.

HARBORNE, J. B. 1963. Plant polyphenols. X. Flavone and aurone glycosides of Antirrhinum. *Phytochem.*, *2*, 327-334.

HARBORNE, J. B. 1967. *Comparative biochemistry of the flavonoids*. Academic Press.

HARRISON, B. J., AND FINCHAM, J. R. S. 1964. Instability at the *pal* locus in *Antirrhinum majus*. I. Effects of environment on frequencies of somatic and germinal mutation. *Heredity*, *19*, 237-258.

SCOTT-MONCRIEFF, R. 1937. Biochemistry of flower colour variation. In *Perspectives in Biochemistry*, Ed. J. Needham and D. E. Green, 230-243. Cambridge University Press.

STICKLAND, R. G., AND HARRISON, B. J. 1973. Precursors and pigmentation in *Antirrhinum majus*. *John Innes Annual Report.*

PRECURSORS AND GENETIC CONTROL OF PIGMENTATION

2. GENOTYPE ANALYSIS OF PIGMENT CONTROLLING GENES IN ACYANIC PHENOTYPES IN *ANTIRRHINUM MAJUS*

B. J. HARRISON and R. G. STICKLAND

John Innes Institute, Colney Lane, Norwich NOR 70F

Received 19.ii.74

SUMMARY

Imbibition of dihydroquercetin, dihydrokaempferol and naringenin by the flowers of certain genotypes of *Antirrhinum majus* enables rapid identification of hypostatic genes involved in pigment synthesis. Induced synthesis of anthocyanidin in acyanic flowers has been shown to occur after imbibition of particular acyanic flower homogenates.

1. INTRODUCTION

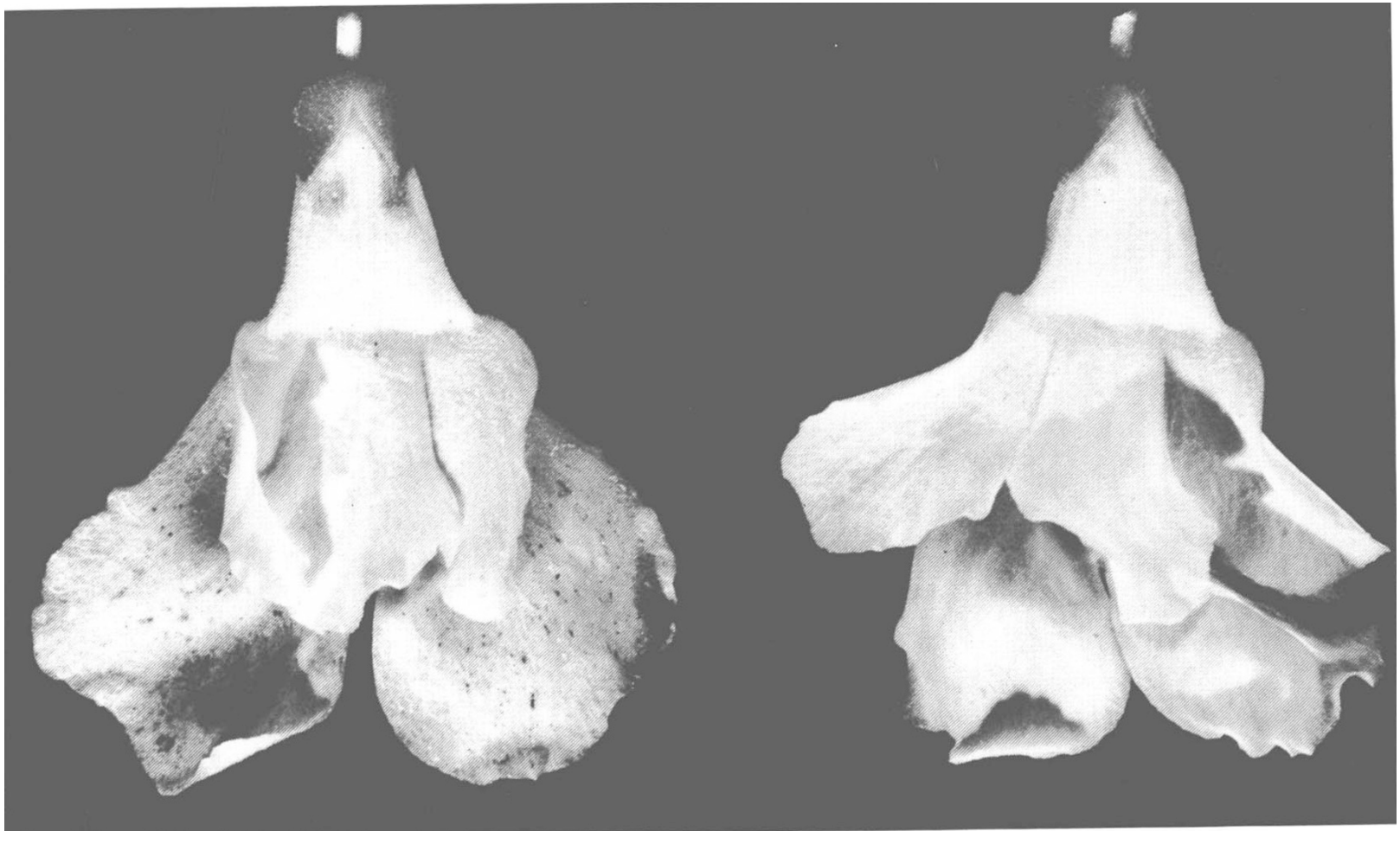
THE pigment pathway in *Antirrhinum majus* culminating in anthocyanidin can be blocked in several places by known genes. It has been found possible to synthesise anthocyanidins in acyanic flowers by administering the flavanonol precursors dihydroquercetin, dihydrokaempferol and dihydromyricetin (Stickland and Harrison, 1974). The presence of homozygous *nivea* (*niv*) in the genotype precludes the identification by visual inspection of the hypostatic genes *incolorata* (*inc*), *delila* (*del*), *pallida* (*pal*) alleles and *eosinea* (*eos*) except by breeding techniques; however, the use of these precursors and the flavanone, naringenin, has enabled many genotypes containing these genes to be readily identified.

2. MATERIALS AND METHODS

With the exception of naringenin (5,7,4'-trihydroxy flavanone) obtained from Koch-Light Laboratories Ltd. and used as a saturated aqueous solution all other materials and methods are as described by Stickland and Harrison (1974). The homogenates were prepared from macerated and ground

Plate I

Left. Untreated corolla of homozygous *incolorata*:*pallida-recurrens.*
Right. Similar corolla treated with dihydroquercetin. The mutant sites of *pallida-recurrens*→ *Pallida* are indicated by the flakes of synthesised cyanidin.



flower material to which approximately 10 per cent distilled water was added. The unfractionated homogenate was administered to the cut flowers by immersion of the corolla tube in a similar way to that adopted for chemical imbibition.

3. Results and conclusions

The genotype *niv*:*inc* (or *Inc*):*Pal* is phenotypically *niv*; the imbibition of dihydroquercetin (DHQ) initiates cyanidin synthesis (fig. 1, 1) and this

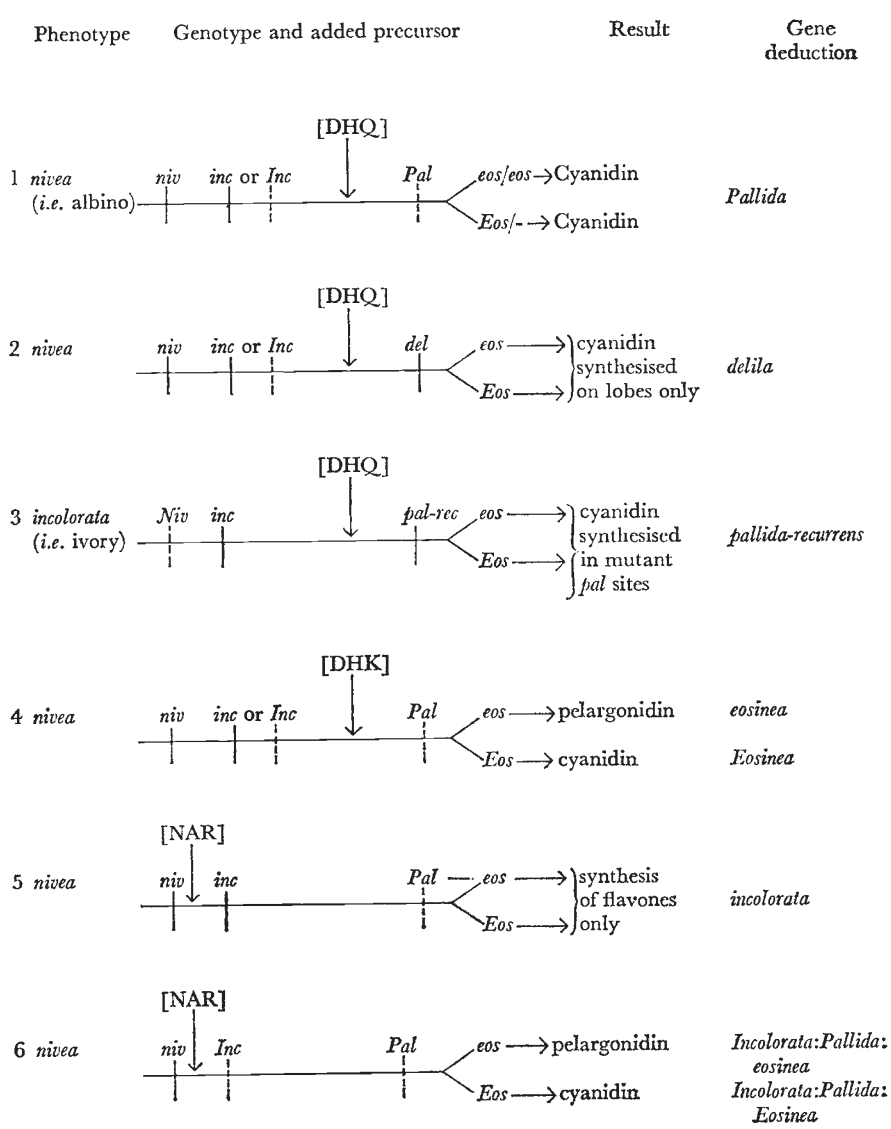


Fig. 1.—Abbreviations: [DHK], [DHQ] and [NAR], point of administration of dihydrokaempferol, dihydroquercetin or naringenin respectively. Gene names are given in full in the last column. Genetic blocks and functional genes are indicated by solid and broken upright bars respectively.

synthesis occurs in both *inc* and *Inc* plants provided that the plant is *Pal.* It is, therefore, possible to deduce the presence of the dominant *Pal* component. The DHQ precursor initiates cyanidin only, irrespective of whether the plant is *eos* (gene for pelargonidin) or *Eos* (gene for cyanidin). The use of the precursor dihydrokaempferol (DHK) enables the identification of the *eos/eos* genotype since flowers bearing the *Eos* allele can add a 3-hydroxyl group to form cyanidin (fig. 1, 4).

Homozygous *del* flowers have no anthocyanidin in the corrola tube. The imbibition of DHQ in an acyanic genotype that is also *del* causes synthesis of anthocyanidin only in the lobes, the tube remaining acyanic (Fig. 1, 2).

Some *pallida* alleles can simulate the effect of *inc*; for example, *pal-tincta* is usually acyanic and a *pal-tubocolorata* plant that is also *del* is not recognisable since the ring of pigment on the base of the corolla tube is unexpressed; a *pallida-recurrens* (*pal-rec*) plant that is not somatically mutating is also acyanic (Harrison and Fincham, 1964). The imbibition of DHQ on a flavone producing plant (*i.e. Niv*) produces cyanidin if *inc*:*Pal* but no cyanidin is synthesised if the block is due to a *pal* allele.

The mutability of the unstable *pal-rec* allele has been shown to be highly sensitive to temperature during flower development (Harrison and Fincham, 1964) and to a genetic stabiliser system (Harrison and Fincham, 1968). The possibility of genetic blocks in the pigment pathway as an influence on the genetic instability was also considered. The genotype *Niv*:*inc*:*pal-rec* is normally unable to express mutations of *pal-rec* to *Pal* because of the acyanic block of *inc*. However, the imbibition of DHQ by such a genotype enables recognition of somatic mutations in corolla tissue (fig. 1, 3). Plate I shows the effect of DHQ on the otherwise acyanic corolla and the readily identifiable mutant areas. The incorporation of DHQ has thus conclusively shown that mutations of *pal-rec* can occur somatically in a plant whose pigment pathway is genetically blocked; however, it does not, at this stage, invalidate the, perhaps, remote possibility that the frequency of mutations may be affected.

The incorporation of the analogue of apigenin, naringenin, into the earlier part of the pigment pathway permits the identification of *inc* in a *niv* plant (fig. 1, 5). The *inc* allele only blocks the further development of flavanone to flavanonol, and synthesis of flavone, which can easily be identified chromatographically, follows the imbibition of naringenin. Naringenin, in addition to the use of DHK, enables the recognition of the *eos* versus *Eos* constitution (fig. 1, 6) in a *niv*:*Inc*:*Pal* plant. Pelargonidin is synthesised in an *eos/eos* genotype while in an *Eos*/$^{-}$ genotype cyanidin is produced.

In addition to the chemical precursors mentioned above, natural precursors obtained from macerated and ground flowers have initiated the synthesis of anthocyanidin. A homogenate of *Niv*:*Inc*:*Pal*:*eos* (a pelargonidin producing plant) induces the formation of cyanidin in *Niv*:*inc*:*Pal*:*Eos* (an acyanic plant in which cyanidin is blocked by *inc*). This is comparable to the initiation of cyanidin synthesis by DHK (the precursor for pelargonidin) in this genotype. This technique enables the recognition of the constitution in respect of the *eos* locus of the recipient plant. A homogenate of genotype *Niv*:*inc*:*Pal* was administered to *niv*:*Inc*:*Pal* and a low level of anthocyanidin synthesis occurred after two days (fig. 2). As no precursor would be in the latter genotype that was not also present in the recipient

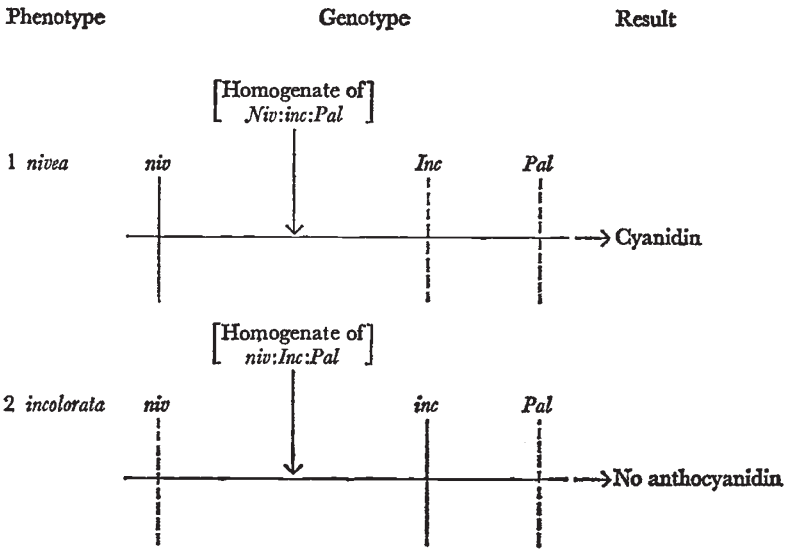


FIG. 2

the reciprocal treatment would be expected to be negative. This prediction was confirmed. Thus from two acyanic stocks of different genetic constitution a reciprocal difference in pigment release was observed from the use of homogenised flower material. It was possible from this result to deduce that the material containing the epistatic *niv* gene was also carrying *Inc*, as otherwise no anthocyanidin would be synthesisable.

4. REFERENCES

HARRISON, B. J., AND FINCHAM, J. R. S. 1964. Instability at the *pal* locus in *Antirrhinum majus*. 1. Effects of environment on frequencies of somatic and germinal mutation. *Heredity*, *19*, 237-258.

HARRISON, B. J., AND FINCHAM, J. R. S. 1968. Instability at the *pal* locus in *Antirrhinum majus*. 3. A gene controlling mutation frequency. *Heredity*, *23*, 67-72.

STICKLAND, R. G., AND HARRISON, B. J. 1974. Precursors and genetic control of pigmentation. 1. Induced biosynthesis of pelargonidin, cyanidin and delphinidin in *Antirrhinum majus*. *Heredity*, *33*, 108-112.