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Are Allozymes Differing in Substrate Specificity Involved in the Evolution of *Silene* Species?

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Summary. The concerted action of two flavone-skeleton modifying genes, *P* and *Me*, and the alleles of three independently segregating loci *g*, *gl* and *fg* involved in flavone-glycosylation lead to the 33 different flavones so far identified in *Silene*. The alleles of the different loci involved in flavone-glycosylation control enzymes which differ in substrate specificity, a phenomenon not often described in higher organisms. The alleles of the different loci are variously distributed over the different species. The possible evolutionary implications of these distributions are discussed.

Key words: Allozymes – Differing substrate specificity – Flavonoids – Flavonoid-glycosylation – Gene evolution – Silene species

Introduction

Our insight into the mechanisms leading to new metabolic functions in microorganisms has greatly increased during the last few years. Appropriate selection has been used to isolate strains of micro-organisms adapted to growth on compounds not usually encountered in the natural environment. Such adaptation can be effected by various mechanisms, for example:

1) mutation of the regulator gene, resulting in constitutive synthesis of an enzyme with poor activity for the novel substrate (Clarke 1974; 1981; Lin et al. 1976),

2) duplication of the structural gene increasing the amount of constitutive enzyme present (Hartley 1974),

3) mutations in the structural gene itself giving rise to enzymes able to utilise the novel substrate (Clarke 1974, 1981; Mortlock 1976, 1982) or

4) recruiting of silent regions of the bacterial genome, remnants of past metabolic activites retained in an almost inert form and providing a reservoir of genetic material to be drawn upon if required (Hall 1978).

The interaction of plasmids, insertion elements and transposons account for the rapid spread of adaptive properties through the bacterial populations.

In higher organisms analogous processes of biochemical adaptation must play a role, but our knowledge in this field is only fragmentary. The rather static environment in the cell of higher organisms appears to preclude strong changes in the interaction within and between the primary biochemical pathways. In higher plants, apart from morphological adaptations, the biochemical potency for synthesizing secondary compounds is of importance for the interaction of the plant with its environment. We may therefore assume that the biochemical pathways leading to the secondary compounds are much more prone to change as a result of constraints put upon them by environmental factors. The pathways leading to the main group of secondary compounds, the "plant phenolics" among which the flavonoids are the most important representatives, appear especially to be involved in the interaction of the plant with its environment (McClure 1975). In this paper the genetic mechanisms controlling flavonoid variation in *Silene* are described and it is shown that much of this variation is the result of allozymes which differ in substrate specificity.

Material and Methods

The plant material was either grown in our experimental plot or in greenhouses. The seed of *Silene diclinis* and *S. marizii* was provided by Dr. H. C. Prentice and the seed of *S. heuffelii* was provided by the botanical garden of Cluj, Romania. The flavonoids of the three endemic European species of section *Elisanthe* from the genus *Silene, S. diclinis, S. heuffelii* and *S. marizii,* were identified as described by van Brederode and van Nigtevecht (1972). Biochemical tests of the glycosyltransferases were done according to van Brederode and van Nigtevecht (1974a). Old data for *S. pratensis* and *S. dioica* were compared with the new results from the endemic species.

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Results and Discussion

The flavones present in five species of the section *Elisanthe* from the genus *Silene* are all glycosides of the basic flavone isovitexin (6-C-glucosyl-apignin) and its 3′-OH and 3′-OMe derivatives, isoorientin and isoscoparin (Fig. 1). The presence of the two latter compounds is restricted to the green parts and is dependent on high light levels (> 20,000 lux). Hydroxylation of the 3′-position of isovitexin is controlled by gene P. For the 3′-OH methylating activity (*Me*) no recessive allele has been identified so far; this activity is always present (Brederode and Kamps-Heinsbroek 1982). In mature plants and in the petals the glycosylation patterns of the flavones are determined by the presence of alleles from three different independent loci: *g*, *gl* and *fg* (Brederode et al. 1980; Mastenbroek et al. 1982) (Table 1). For these loci three, three and two alleles respectively are known. The dominant alleles of the *g*-locus are *g*G* and *g*X*, which bind glucose and xylose respectively to the free 7-OH group of isovitexin; the recessive *g* is the third allele (Brederode and Nigtevecht 1972, 1974a). The recessive allele of the *gl*-locus is *gl* and the two dominant alleles are *gl*R* and *gl*A*. The two dominant alleles control the binding of rhamnose and arabinose respectively to the 2″-OH group of isovitexin (Besson et al. 1979). For the *fg*-locus only two alleles have been identified: *fg*, the recessive, and *Fg*, the dominant, which controls the binding of glucose to the 2″-OH group of isovitexin (Brederode and Nigtevecht 1974b). In the five different *Silene* species that were investigated dominant alleles were always found at the *g* and *fg* loci, but a dominant allele at the *gl*-locus was only found in three of the five species (Table 1). This picture is complicated by the fact that the alleles of the different loci are variously distributed among the species. For instance, allele *g*G* of the *g*-locus can be found in all species, but allele *g*X* of the same locus is only found in *S. dioica*. Another example is the allele of the *gl*-locus, *gl*A*, which is present in two of the species (*S. dioica* and *S. diclinis*) that possess dominant alleles at the *gl*-locus but is absent from the third, *S. pratensis* (Table 1).

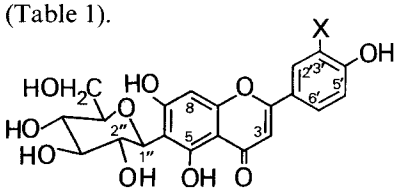

Fig. 1. Structure of isovitexin(X = H), isoorientin(X = OH) and isoscoparin(X = OCH3). The conversion of isovitexin to isoorientin is controlled by gene *P*. The formation of isoscoparin from isoorientin is controlled by gene *Me*. At the 3 loci for flavoneglycosylation *g*, *gl* and *fg*, alleles *gl* A*, *gl* R* and *Fg* substitute respectively arabinose, rhamnose and glucose for 2″OH. Alleles *g* G* and *g* G* substitute respectively glucose and xylose for 7-OH

Table 1. Five *Silene* species from section-*Elisanthe* and the flavone-glycosylation loci and alleles they possess

	Loci		
	g	*gl*	*fg*
S. dioica	g g*G g*X	gl gl*R gl*A	fg Fg
S. pratensis	g g*G	gl gl*R	fg Fg
S. diclinis	g g*G	gl gl*A	fg Fg
S. marizii	g g*G		fg Fg
S. heuffelii	g g*G		fg Fg

The different alleles of these three loci possess another remarkable property. The alleles *g*G* and *g*X* of locus *g* and alleles *gl*A* and *gl*R* of locus *gl* control enzymes which differ in their substrate specificity. Alleles *g*G* and *g*X* control enzymes which catalyze the transfer of glucose and xylose respectively from UDP-glucose and UDP-xylose to the 7-hydroxyl group of isovitexin (Brederode and Nigtevecht 1974a). The alleles *gl*R* and *gl*A* are the structural genes for the enzymes which catalyze the transfer of rhamnose (Brederode, unpublished results) and arabinose (Heinsbroek et al. 1979) from the respective UDP-activated glycosides to the 2″-OH group of the C–C bound glucose of isovitexin on the 6-position. The enzymes are very specific as to the sugar moiety to be transfered and to the hydroxyl-position on the flavone skeleton to be glycosylated. The enzyme controlled by allele *g*G* is not able to catalyze the transfer of xylose (which is controlled by its allele *g*X*) to the 7-hydroxyl position; neither is *gl*A* able to catalyze the *gl*R* controlled transfer of rhamnose to the 2″-hydroxyl group (Brederode and Nigtevecht 1974a, 1974b, Heinsbroek et al. 1979). The phenomenon of alleles controlling allozymes which differ in their substrate specificity is quite unique, despite the large genetic variation demonstrated to be in higher organisms.

The introduction of electrophoretic and histochemical techniques in the study of genetics revealed much hidden variation in higher organisms (e.g. Lewontin 1974). Variations in substrate specificity, however, cannot easily be detected by these techniques. Therefore, an allele with an altered substrate specificity will be scored as a "null allele". Consequently, the phenomenon of differences in substrate specificity between enzymes which are controlled by various alleles of a single locus might be rather common, but easily escape detection. One of the best known examples are the human bloodgroup alleles *IA*, *IB* and *I*. Allele *IA* controls an N-acetylgalactosaminyltransferase, allele *IB* a galactosyltransferase. Both enzymes transfer the appropriate hexose to galactose at the reducing end of the H-active structure and form alpha 1–3 linkages. The third allele is inactive and does not produce a functional glycosyltransferase (Ginsburg 1972). There is circumstantial evidence for selection operating on the *I* locus, although other mechanisms may be involved as well (Cavalli-Sforza and Bodmer, 1971). For the aliesterase locus in the

housefly, the situation is more straightforward. Oppenoorth (1965) demonstrated that a "null allele" of the aliesterase locus in a resistant race of the housefly was able to hydrolyse an organophosphor-insecticide. In this case one may assume that the difference in substrate specificity reflects a response to the selection pressure caused by the insecticide.

It is remarkable that the modification of the flavones leading to the 33 different flavones, now identified in *Silene,* is the result of the concerted action of two genes that modify the flavone-skeleton and of alleles at three independently segregating loci that control enzymes which differ in substrate specificity with regard to the sugars they transfer. It appears that *Silene* possesses a flexible system in which a few loci are responsible for a large amount of flavone variation. This systems is reminiscent of the mechanisms responsible for the variation in mammalian antibodies.

The distribution of the various alleles over the five *Silene* species presents us with an interpretative problem. Are we observing adaptive differences between the species or chance relicts from earlier divergence events? Since we are working with closely related species we can also ask about the evolutionary relationships among the species. There may conceivably be some link between the ability to make anthocyanins and the presence of allele *gl*A* at the *gl*-locus. This allele is only found in the two species with red petals (*S. dioica* and *S. diclinis*), all the other species having white petals. Also, it is only in *S. dioica* that all alleles can be found. This suggests that *S. dioica* is the oldest of the species (or at least stands closest to a hypothetical ancestor). If so, the other species evolved by losing genes! On the other hand *S. dioica* might recently have evolved along an evolutionary pathway during which the new genes resulted by gene-duplication. The five *Silene* species we are investigating present us thus with an unique system in which it will be possible to take an experimental approach to the role of allozymes with different substrate specificities in evolution.

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