O7g and *D6a:* Two Flavone Glycosylating Genes in *Silene,* which are Only Expressed in Cotyledons and Rosette Leaves

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Two further loci can be added to the three already known (*g*, *gl* and *fg*) in *Silene* controlling the 7-O and 2″-O-glycosylation of the C-glycosylflavone isovitexin (6-C-glucosylapigenin). These loci, *O7g* for the 7-O-galactosylation and *D6a* for the 2″-O-arabinosylation, appear only to be expressed in cotyledons and rosette leaves and control the biosynthesis of isovitexin 7-O-galactoside and isovitexin 7-O-galactose 2″-O-arabinoside in these parts of plants in which the recessive alleles of the loci *g*, *gl* and *fg* are homozygous (Steyns *et al.* [9]).

The possibility that locus *g*, whose dominant alleles *gG* and *gX* control the 7-O-glucosylation and the 7-O-xylosylation of isovitexin respectively, controls the synthesis of isovitexin-7-O-galactoside was excluded by demonstrating the presence of isovitexin-7-O-galactoside and the 7-O-galactosyltransferase catalyzing its biosynthesis in addition to isovitexin-7-O-glucoside and the 7-O-glucosyltransferase in cotyledons of *gGgG* seedlings.

The synthesis of the 2″-O-arabinoside is neither controlled by locus *gl*, whose dominant alleles *glR* and *glA* control the 2″-O-rhamnosylation and 2″-O-arabinosylation of isovitexin respectively, nor by locus *fg*, whose dominant allele *Fg* controls the biosynthesis of isovitexin 2″-O-glucoside. This was shown by the presence of isovitexin-7-O-galactose 2″-O-arabinoside and the 2″-O-arabinosyltransferase catalyzing the synthesis of the 2″-O-arabinoside in addition to isovitexin-7-O-galactose 2″-O-rhamnoside in cotyledons of *glRglR* seedlings and by the presence of isovitexin-7-O-galactose 2″-O-arabinoside and the 2″-O-arabinosyltransferase in addition to isovitexin-7-O-galactose 2″-O-glucoside and the 2″-O-glucosyltransferase in cotyledons of *Fg Fg* seedlings.

Introduction

The glycosylation of the C-glycosylflavone isovitexin (6-C-glucosylapigenin) in the petals of the two closely related *Silene* species *S. pratensis* and *S. dioica* is controlled by the action of three independently segregating loci (Fig. 1). The locus *g* is responsible for the attachment of sugars to the 7-OH in the A-ring of the isovitexin molecule, whereas the loci *gl* and *fg* are involved in the glycosylation of the 2″-OH of the 6-C-bound glucose. Three alleles are known for the loci *g* and *gl*, whereas only two have been identified for the *fg* locus. The dominant alleles of the *g* locus, *gG* and *gX*, code for glycosyltransferases that bind glucose and xylose respectively to the 7-OH (Brederode & Nigtevecht [1, 2]). The dominant alleles of the loci *gl* and *fg*, *glA*, *glR* and *Fg*, control the binding of arabinose, rhamnose and glucose respectively to the 2″OH (Heinsbroek *et al.*; Besson *et al.*; Brederode & Nigtevecht [3–5]). In the presence of the recessive alleles no sugars are bound to the isovitexin molecule and consequently only isovitexin itself is found in the petals (Brederode and Nigtevecht [6]). The glycosyltransferases encoded by the various alleles are very specific for the sugar to be transferred to isovitexin. Thus we are dealing here with the quite unique phenomenon of alleles controlling allozymes differing in substrate specificity (Brederode and Mastenbroek [7]). In this respect the flavone glycosylation in *Silene* is reminiscent of the human blood group ABO system, in which the alleles A and B code for an N-acetylgalactosaminyltransferase and a galactosyltransferase respectively, allele O being the recessive (for a recent review see Yoshida [8]).

The 7-O and 2″-O-glycosides of isovitexin are present in the vegetative parts of the plants as well. Surprisingly, however, in the vegetative parts of plants with the recessive alleles of the loci *g*, *gl* and *fg* (genotype *gg glgl fgfg*) we also found isovitexin-O-glycosides. These glycosides are present

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only in the cotyledons and the rosette leaves, whereas the stem leaves and petals of these so-called isovitexin plants only accumulate the aglycone isovitexin (Steyns *et al.* [9]). So either the synthesis of the flavone glycosides is differentially regulated or the glycosylation of isovitexin in the isovitexin plants is controlled by loci different from the loci *g*, *gl* and *fg*. In this paper the experiments to resolve this problem are presented.

Experimental

Plant material

Seeds of the various genotypes were sown on earth in pots in our greenhouse. Cotyledons were harvested about 14 days later, before the first leaflet had fully developed.

Flavone extraction and purification

The flavones in the plant organs were obtained by repeated extraction with 70% aqueous methanol (cotyledons, petals) or aceton (rosette leaves). The flavones in the methanolic extracts were concentrated under reduced pressure with a rotary evaporator and subsequently purified by paper chromatography. The aceton extract was evaporated to dryness with a rotary evaporator, the residue taken up in 70% aq. MeOH and extracted several times with chloroform in a separation funnel. The aqueous layer was concentrated and the flavones in it purified by paper chromatography.

Routinely the flavones were first separated on Whatmann-3 paper with BAW (butanol-acetic acid-water,4-1-5, upper phase) as solvent. Flavone bands were cut out, eluted with MeOH and then chromatographed on Wh-3 with water. Occasionally a third purification step was performed on Wh-3 using 15% acetic acid (HAc) in water. Flavone referents were extracted from petals (IV7G6A, IV7G6R, IV7G6G, IV7G, IV7X, IV6A, IV6R, IV6G) or rosette leaves (IV7Gal6R, IV7Gal6G) of plants of the appropriate genotype. IV7Gal and IV7Gal6A were extracted from rosette leaves of IV plants. Refer to Fig. 1 and Tables for explanation of flavone abbreviations.

Flavone structure elucidation

UV spectrum analysis was done according to Mabry *et al.* [10]. Flavones were hydrolyzed completely in 20% MeOH/1 N HCl at 100 °C in 2 h. Partial acid hydrolysis was achieved by hydrolyzing 5–10 min under the same conditions. The aglycone(s) were then extracted with water saturated butanol; the aqueous layer with the liberated sugars was freed from HCl by Ionenaustauscher V (Merck). Sugars and aglycone(s) were then identified by co-chromatography with known referents. The sugars galactose, glucose, arabinose, xylose and rhamnose are clearly separated on the following thin layer chromatograms: 1. silica 60 (Merck Fertigplatten)/aceton-water 9:1; 2. silica 60/ethylacetate-pyridine-water-methanol 80:20:10:5 (EWPM); 3. cellulose (Merck Fertigplatten)/ethylacetate-pyridine-acetic acid-water 36:36:7:21. Sugars are detected by spraying with aniline biphthalate (2.5 g/100 ml), followed by heating at 110 °C for several minutes. Flavone aglycones are separated routinely by paper chromatography using Schleicher & Schull paper (46 × 56 cm, 2043a) and BAW and 1% HCl in water as solvents. Thin layer chromatography is performed with cellulose plates (Merck Fertigplatten) and BAW, 1% HCl or 15% HAc or with silica 60/EWPM.

Enzyme assays

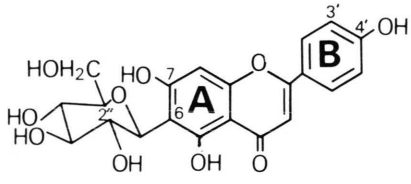
Cotyledons of the various genotypes were homogenized freshly or after storage at −20 °C in 50 mM Na_2HPO_4/KH_2PO_4 buffer pH 7.0, 20 mM 2-mercaptoethanol, 0.01% triton X-100, 5% PVP-44 000 in an all glass Potter-Elvehjem homogenizer at 0 °C. The homogenate was centrifuged at 38 000 × *g* for 25 min. The supernatant was used to test for the presence of the various flavone O-glycosyltransferases. Enzyme activity was tested at 30 °C during 30 min. The standard reaction mixture consisted of 25 µl 38 000 × *g* supernatant, 2 µl flavone and 2 µl UDP-glycoside (for concentration of substrates see Tables I, III, IV and V). The reaction was stopped by addition of 10 µl 20% TCA in methanol. The denaturated protein was pelleted and the supernatant was applied onto Wh-3 paper together with carrier flavone(s). The flavones were chromatographed two-dimensionally, first in BAW, then in 1% HCl. The chromatogram allows the distinction of the 7-O-glycosides, 2″-O-glycosides and 7-O,2″-O-diglycosides respectively. Clear and reliable differences between the various diglycosides cannot be obtained in this way, neither can IV7G and

IV7Gal nor IV6R and IV6A be distinguished; however, IV7X can be distinguished from IV7G/IV7Gal and IV6G from IV6R/IV6A. The flavone spots of interest were cut out and the amount of radioactivity in them was counted in a Packard Liquid Scintillation Analyzer using Lipoluma as scintillation liquid. The protein content of the crude homogenate was estimated by the BIO-RAD protein assay (Bradford (11); bovine gamma globuline as standard) and varied between 3 and 6 mg/ml.

Chemicals

Uridine diphospho glucose (UDP-glucose; 233 Ci/mol) and uridine diphospho galactose (UDP-galactose; 340 Ci/mol) were obtained from the Radiochemical Centre, Amersham. Uridine diphospho arabinose (UDP-arabinose; 183 Ci/mol) was supplied by New England Nuclear. Carrier UDP-glucose and UDP-galactose to adjust the specific activity were obtained from Sigma. Polyvinylpyrrolidone (PVP; MW approx. 44000) was obtained from BDH Chemicals Ltd.

Fig. 1: Genetics of the flavone glysolation in the petals.



Isovitexin (IV)

locus	acting on	allele	sugar bound	flavone
g	7OH	g	-	IV
		g^G	glucose	IV7G
		g^X	xylose	IV7X
gl	2"OH	gl	-	IV
		gl^R	rhamnose	IV6R
		gl^A	arabinose	IV6A
fg	2"OH	fg	-	IV
		Fg	glucose	IV6G

Fig. 1. The "aglycone" isovitexin will be denoted as IV. For reasons of convenience flavones with a sugar bound to the 2"OH are called 6-glycosides; thus isovitexin-2"-O-rhamnoside will be abbreviated as IV6R. The concerted action of the various alleles leads to the synthesis of various diglycosides (*e.g.* in the presence of *gG* and *glR* isovitexin-7-O-glucose 2"-O-rhamnoside (IV7G6R)) is synthesized.

Results and Discussion

The genetics of the flavone glycosylation in the petals of *Silene pratensis* and *S. dioica* is shown in Fig. 1. From this figure it can be deduced that the genotype *gg glgl fgfg* is not able to glycosylate isovitexin and consequently, petals of plants of this genotype only accumulate isovitexin (IV). In cotyledons and rosette leaves of these so-called isovitexin plants (IV plants), however, we detected IV-7-O-galactoside (IV7Gal) and IV-7-O-galactose 2"-O-arabinoside (IV7Gal6A) (Steyns *et al.* [9]). It was subsequently shown by Brederode and Steyns [12]) that in crude homogenates of the cotyledons an enzyme activity was present that was able to synthesize IV7Gal from IV and UDP-galactose. Additionally, as shown in Table I, in cotyledons of IV plants an enzyme activity capable of synthesizing isovitexin 2"-O-arabinoside (IV6A) from IV and UDP-arabinose is present as well. This enzyme activity does not recognize IV7Gal as a substrate. Table I also demonstrates that the galactosyltransferase activity can be monitored much more readily using IV6A rather than IV as a substrate (1420 cpm in IV7Gal6A, 220 cpm in IV7Gal), due to differences in K_m for UDP-galactose (J. M. Steyns *et al.*, in preparation). This suggests that *in vivo* the biosynthesis if IV7Gal6A probably proceeds *via* the following pathway: IV → IV6A → IV7Gal6A. The low amount of counts found in IV7Gal6A upon labeling with UDP-glucose probably is the result of an epimerase activity present in the crude homogenate, which converts UDP-glucose to UDP-galactose (Kamps-Heinsbroek, unpublished). The galactosyltransferase also is capable of synthesizing the IV-7,2"-O-diglycosides from the 2"-O-glycosides IV6R and IV6G (not shown).

To determine whether the attachment of galactose to the 7-OH is controlled by the locus *g*, and whether the attachment of arabinose to the 2"-OH is controlled either by locus *gl* or locus *fg*, we reasoned that if these assumptions were correct, it would be impossible to detect isovitexin-7-O-galactoside (IV7Gal) in plants with a genotype with two dominant alleles at the *g* locus (f.i. *gGgG*) and to detect isovotexin-2"-O-arabinoside (IV6A) and/or isovitexin-7-O-galactose 2"-O-arabinoside (IV7Gal6A) in plants with two dominant alleles at the *gl* locus (f.i. *glRglR*) or the *fg* locus (*FgFg*).

Table I. Flavone-O-glycosyltransferase activities present in cotyledons of genotype *gg glgl fgfg*. IV7Gal and IV7Gal6A stand for IV-7-O-galactoside and the 2″-O-arabinoside of it respectively; for other flavone symbols refer to Fig. 1 (0) means that the counted value (cpm, counts per minute) does not exceed the background value (20 cpm). (−) means "not counted". The concentrations of the substrates: IV, IV6A and IV7Gal 0.21 mM; UDPgalactose (0.84 Ci/mol) 1.1 mM; UDPglucose (0.84 Ci/mol) 0.83 mM; UDParabinose (183 Ci/mol) 2.6 μM.

Flavone acceptor	Sugar donor	cpm in flavone carrier				
		IV7G/IV7Gal	IV7X	IV6G	IV6A	IV7Gal6A
IV	UDP-galactose	220	0	0	0	−
IV	UDP-glucose	0	0	0	0	−
IV6A	UDP-galactose	0	−	−	−	1420
IV6A	UDP-glucose	0	−	−	−	80
IV	UDP-arabinose	0	0	0	1670	−
IV7Gal	UDP-arabinose	−	−	−	−	0

Table II. Thin layer chromatography (TLC) of the flavones present in cotyledons of *gGgG glgl fgfg* seedlings (52G). For TLC systems and symbols for flavone referents refer to Mat. & Meth. and Fig. 1/Table I respectively. The 52G flavone bands A and B were purified by paper chromatography using Whatmann III paper and BAW and water as solvents (see Mat. & Meth.); band A appears to be a mixture of two 7-O-glycosides.

	TLC-solvent [R_f-value × 100]			
	BAW	1% HCl	15% HAc	EPWM
52G: band A	46	16	57	27 + 33
52G: band B	45	49	83	22
Flavone referents				
IV7G	47	18	59	33
IV7Gal	46	16	57.5	27
IV7X	51.5	15	55	38
IV7G6A	47	50	83.5	25
IV7Gal6A	46.5	49	83	22

i. Is the synthesis of isovitexin-7-O-galactoside controlled by locus g?

The seedlings originating from cross 52G (genotype *gGgG glgl fgfg*) were screened for the flavones present in their cotyledons. 36 Individuals were investigated chromatographically: all cotyledons exhibited the same flavone pattern. We therefore pooled the cotyledons of 52G seedlings, purified the flavones and elucidated the flavone structure by means of cochromatography with known referents and acid hydrolysis.

Table II shows the presence of two isovitexin-7-O-glycosides and one isovitexin-7,2″-O-diglycoside in the cotyledons of the *gGgG* seedlings. The chromatographic results suggest the presence of isovitexin-7-O-glucoside (IV7G) and IV7Gal in addition to IV7Gal6A. This was corroborated by acid hydrolysis: the mixture of 7-O-glycosides yields IV as aglycone and galactose and glucose as released sugars, whereas the diglycoside yields IV and galactose and arabinose. Partial hydrolysis of the diglycoside showed that the 7-O-glycoside formed comigrates with IV7Gal. The 2″-O-arabinoside (IV6A) cannot be obtained by partial acid hydrolysis, because arabinose is liberated very quickly (Steyns *et al.* [9]).

Table III shows the presence of the various flavone O-glycosyltransferases in crude homogenates of cotyledons of *gGgG* seedlings. The presence of a 7-O-galactosyltransferase is clearly shown by offering IV6A as a substrate. As this transferase is capable of recognizing IV as well, the counts found at the 7-O-glycoside position upon labeling with UDP-glucose might originate from IV7Gal rather than from IV7G due to epimerase activity in the homogenate. Acid hydrolysis of the labeled 7-O-glycoside, however, revealed that the counts

Table III. Flavone-O-glycosyltransferase activities present in cotyledons of *gGgG glgl fgfg* seedlings (52G). For symbols refer to Fig. 1/Table I. The concentrations of the UDP-glycosides were the same as in Table I, whereas 0.14 mM of IV or IV6A were present.

Flavone acceptor	Sugar donor	cpm in flavone carrier		
		IV7G/ IV7Gal	IV6A	IV7Gal6A/ IV7G6A
IV	UDP-glucose	310	−	0
IV	UDP-galactose	310	−	10
IV6A	UDP-glucose	5	−	90
IV6A	UDP-galactose	0	−	1010
IV	UDP-arabinose	−	350	−

originated from glucose and not from galactose. We can therefore conclude that both a 7-O-galactosyl and a 7-O-glucosyltransferase are present in the *gGgG* cotyledons. As expected from the presence of IV7Gal6A an 2″-O-arabinosyltransferase activity can be demonstrated as well (Table III).

Comparable results were obtained with cotyledons of *gGgG glgl fgfg* seedlings of two other crosses. We must conclude therefore that locus *g* does not control the attachment of galactose to the 7-OH of isovitexin.

It can be argued that the genetics of the flavone glycosylation remains valid if we assume that locus *g* regulates the presence of the glycosyltransferases rather than codes for proteins with transferase activity. But then it must be assumed that the genes that code for the transferase activities are always present. To account for the Mendelian inheritance observed (Brederode and Nigtevecht [2, 6]), at least three alleles of the putative regulatory locus *rg* must be hypothesized. The first two alleles then control the presence of the 7-O-glucosyl (*rg-glc*) and the 7-O-xylosyltransferase (*rg-xyl*), whereas the third allele controls the presence of the 7-O-galactosyltransferase (*rg-gal*). Differences in metabolic environment can explain that in IV plants the flavones IV7Gal and IV7Gal6A only accumulate in cotyledons and rosette leaves but not in stem leaves and petals (Steyns *et al.* [9]). In this model the genotype of IV plants is *rg-gal/rg-gal* and consequently, it would be impossible to detect 7-O-galactosides in genotype *rg-glc/rg-glc*. However, this does not prove to be so (Tables II and III). The conclusion must remain therefore that the coupling of galactose to the 7-OH of IV is controlled by a locus different from locus *g*.

ii. Is the synthesis of isovitexin-2″-O-arabinoside controlled by the loci gl or fg?

Using a similar approach and an analogous argumentation we can conclude that neither locus *gl* (Table IV) nor locus *fg* (Table V) controls the coupling of arabinose to the 2″-OH of IV in cotyledons and rosette leaves of IV plants.

The results can be summarized as follows:

– in cotyledons of *gg glRglR fgfg* seedlings a mixture of two diglycosides was found; acid hydrolysis yields IV as the aglycone and galactose, arabinose and rhamnose as the liberated sugars; the two diglycosides comigrate with IV7Gal6A and IV7Gal6R respectively; in a crude cotyledon

Table IV. Thin layer chromatography (TLC) of flavones (I) and activities of flavone-O-glycosyltransferases (II) present in cotyledons of *gg glRglR fgfg* seedlings (50D). For symbols refer to Fig. 1 and Tables I/II. The concentrations of the substrates: IV, IV6A 0.21 mM; UDPgalactose (340 Ci/mol) 3.2 μM; UDPglucose (233 Ci/mol) 3.2 μM; UDParabinose (183 Ci/mol) 2.6 μM.

I Flavones	TLC-solvent [R_f-value × 100]				Products of acid hydrolysis
	BAW	1% HCl	15% HAc	EPWM	
50D: band A	46.5	52.5	83	17 + 22	IV, galactose, arabinose, rhamnose
Flavone referents					
IV7Gal6A	46.5	49	83	22	
IV7Gal6R	46	55	84.5	17	
IV7G6R	48	55.5	85.5	20	
IV6A	66.5	41	76	46	
IV6R	65	48	79.5	40.5	

II Flavone-O-glycosyltransferase activities

Flavone acceptor	Sugar donor	cpm in flavone carrier		
		IV7G/IV7Gal	IV6A	IV7Gal6A/IV7G6A
IV	UDP-arabinose	–	140	–
IV	UDP-galactose	460	–	–
IV	UDP-glucose	50	–	–
IV6A	UDP-galactose	–	–	4110
IV6A	UDP-glucose	–	–	330

Table V. Thin layer chromatography (TLC) of flavones (I) and activities of flavone-O-glycosyltransferases (II) present in cotyledons of genotype *gg glgl FgFg* (14F). For symbols refer to Fig. 1 and Tables I/II. The concentrations of the substrates: IV, IV6A 0.21 mM; UDPglucose (233 Ci/mol) 2.45 μM (1) or 6.45 μM (3); UDPgalactose (340 Ci/mol) 2.45 μM (2) or 6.45 μM (4); UDParabinose (183 Ci/mol) 2.6 μM (5).

I Flavones	TLC-solvent [R_f-values × 100]				Products of acid hydrolysis
	BAW	1% HCl	15% HAc	EPWM	
14F: band A	43.5 + 47	51	83	16 + 22	IV, galactose, glucose, arabinose
Flavone referents					
IV7Gal6A	46.5	49	83	22	
IV7Gal6G	44	52.5	85	16	
IV7G6G	45.5	53	85	21	
IV6G	60	45	79	37	

II Flavone-O-glycosyltransferase activities

Flavone acceptor	Sugar donor	cpm in flavone carrier			
		IV7G/IV7Gal	IV6G	IV6A	IV7Gal6A/IV7G6A
1. IV	UDP-glucose	160	16 490	–	–
2. IV	UDP-galactose	1650	13 855	–	–
3. IV6A	UDP-glucose	0	–	–	450
4. IV6A	UDP-galactose	0	–	–	4050
5. IV	UDP-arabinose	–	–	200	–

homogenate an enzyme activity is present that catalyzes the formation of IV6A from IV and UDP-arabinose; as expected, a 7-O-galactosyltransferase activity can be demonstrated as well.

– in cotyledons of *gg glgl FgFg* seedlings two diglycosides, comigrating with IV7Gal6A and IV7Gal6G, were present; acid hydrolysis of a mixture of the diglycosides liberates galactose, glucose and arabinose; partial hydrolysis yielded IV6G and IV7Gal; in crude homogenates of the cotyledons three flavone O-glycosyltransferases were present, catalyzing the synthesis of IV6G, IV6A and IV7Gal6A respectively (the nature of the labeled sugar in the flavone was verified by acid hydrolysis).

In this paper it is demonstrated that in *Silene* two further isovitexin glycosylating loci can be added to the three loci *g*, *gl* and *fg* already known. The expression of locus *O7g*, which controls the 7-O-galactosylation, and of locus *D6a*, which controls the 2″-O-arabinosylation, seems to be restricted to early ontogenetic stages (cotyledons and rosette leaves: Steyns *et al.* [9]). Remarkable is the finding that 1. the biosynthesis of isovitexin-2″-O-arabinoside is controlled by two different genes (*glA*, *D6a*); 2. the glycosylation pattern of isovitexin is controlled by genes, whose expression is dependent on the developmental stage (f.i. the synthesis of isovitexin-7-O-galactoside, controlled by gene *O7g*, is shut off in stem leaves and petals, whereas the synthesis of isovitexin-7-O-glucoside, controlled by gene *gG*, continues). These features make *Silene* a very suitable object for the study of eukaryotic gene regulation.

The genes *O7g* and *D6a* always seem to be present in *S. pratensis* and *S. dioica*. Hence it is not possible to select for recessives of *O7g* and *D6a* in the same way as for the alleles of the loci *g*, *gl* and *fg*. In the light of the deleterious effect of isovitexin on upper epidermal cells of petals of *S. pratensis* plants (Brederode *et al.* [13]), it can be expected that a strong selection exists against plants completely unable to glycosylate isovitexin. One possibility to select for recessives of *O7g* and *D6a* might be to look for their presence in the endemic *Silene* species of the section Elisanthe of *Silene* in Europe. These endemics (*S. marizii*, *S. diclinis*, and *S. heuffelii*) have been shown to be closely related to *S. pratensis* and *S. dioica* (Prentice [14]). Apart from the possibility of finding recessives of *O7g* and *D6a*, the results of the studies on the distribution of the

isovitexin glycosylating genes over these species may contribute, together with data on petal flavonoids (Mastenbroek *et al.* [15]) and various morphological characters (Prentice; Mastenbroek [16–18]), to the reconstruction of the evolution of the *Silene* species in the section Elisanthe (see also Brederode and Mastenbroek [7]).

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[1] J. v. Brederode and G. v. Nigtevecht, Molec. Gen. Genet. **122,** 215–229 (1973).
[2] J. v. Brederode and G. v. Nigtevecht, Phytochemistry **13,** 2763–2766 (1974).
[3] R. Heinsbroek, J. v. Brederode, G. v. Nigtevecht, and J. Kamsteeg, Phytochemistry **18,** 935–937 (1979).
[4] E. Besson, A. Besset, M. L. Bouillant, J. Chopin, J. v. Brederode, and G. v. Nigtevecht, Phytochemistry **18,** 657–658 (1979).
[5] J. v. Brederode and G. v. Nigtevecht, Biochem. Genet. **11,** 65–81 (1974).
[6] J. v. Brederode and G. v. Nigtevecht, Molec. Gen. Genet. **118,** 247–259 (1972).
[7] J. v. Brederode and O. Mastenbroek, Theor. Appl. Genet. **64,** 151–153 (1982).
[8] A. Yoshida, Am. J. Hum. Genet. **34,** 1, 1–14 (1982).
[9] J. M. Steyns, G. v. Nigtevecht, G. J. Niemann, and J. v. Brederode, Z. Naturforsch. **38c,** 544–548 (1983).
[10] T. J. Mabry, K. R. Markham, and M. B. Thomas, The systematic identification of flavonoids. Springer Verlag, New York 1970.
[11] M. H. Bradford, Anal. Biochem. **72,** 248–254 (1976).
[12] J. v. Brederode and J. M. Steyns, Z. Naturforsch. **38c,** 549–550 (1983).
[13] J. v. Brederode, H. H. v. Genderen, and W. Berendsen, Experientia **38,** 929–931 (1982).
[14] H. C. Prentice, Bot. J. Linn. Soc. **77,** 203–216 (1978).
[15] O. Mastenbroek, J. J. Knorr, R. Kamps-Heinsbroek, J. W. Maas, J. M. Steyns, and J. v. Brederode, Z. Naturforsch. **38c,** 894–898 (1983).
[16] H. C. Prentice, Bot. J. Linn. Soc. **78,** 181–212 (1979).
[17] H. C. Prentice, Bot. J. Linn. Soc., in press.
[18] O. Mastenbroek, Patterns of variation in European *Silene pratensis,* Thesis, University of Utrecht (1983).