Genetics of aliphatic glucosinolates. III. Side chain structure of aliphatic glucosinolates in *Arabidopsis thaliana*

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The inheritance of aliphatic glucosinolates in *Arabidopsis thaliana* was studied. Analysis of F_3 families from a cross between the ecotype Limburg-5 and the accession H51 (an inbred line derived from the ecotype Stockholm) suggested that alleles at a single locus regulate the conversion of methylsulphinylalkyl glucosinolates into alkenyl (and hydroxyalkenyl) glucosinolates. Likewise, analysis of recombinant inbred lines derived from a cross between the ecotypes Columbia and Landsberg *erecta* suggested that alleles at a single locus regulate the conversion of methylsulphinylpropyl glucosinolate into hydroxypropyl glucosinolate. Both loci mapped to a similar position on chromosome 4 and it is suggested that these alleles occur at the same locus. A genetic model is proposed in which the aliphatic glucosinolates of *A. thaliana* are determined by alleles at three loci. The ecological significance of variation in aliphatic glucosinolates is discussed.

Keywords: *Arabidopsis thaliana*, gene mapping, genetic diversity, glucosinolates, pests.

Introduction

Aliphatic glucosinolates are thioglycosides which occur in the leaves of members of the Brassicaceae and related families. They are important in mediating pest interactions and have been shown to attract specialized cruciferous pests such as flea beetles and to repel generalist herbivores such as slugs and birds. In addition, they determine the feeding quality of rapeseed meal and the flavour of cruciferous vegetables and salad crops. The aliphatic glucosinolate molecule comprises two parts: a common glycone moiety and a variable aglycone side chain derived from methionine (Fenwick *et al.*, 1983). The biological activity of glucosinolates is largely dependent on the structure of the side chain which determines the nature of hydrolysis products which are produced following tissue damage. Intraspecific variation in the types of aliphatic glucosinolates has been shown to occur in *Cakile edentula* (Rodman, 1980), *Brassica oleracea* (*sensu lato*) (Mithen *et al.*, 1987) and *Arabidopsis thaliana* (Bano, 1993). This variation may be in the length of the aliphatic side chain or in the types of substituted groups which it may possess, both of which influence pest interactions

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(Chew, 1988; Mithen 1992; Simmonds *et al.*, 1994). In *A. thaliana*, methylsulphinylalkyl, alkenyl, hydroxyalkyl and hydroxyalkenyl homologues of propyl and butyl glucosinolates are commonly found (Bano, 1993, fig. 1), with trace levels of longer side chain homologues.

In a previous publication, it was shown that the side chain length of aliphatic glucosinolates in *A. thaliana* is regulated by alleles at a single locus on chromosome 5 (Magrath *et al.*, 1994). In the present study, we consider two major types of side chain modifications. Firstly, a desaturation reaction which converts methylsulphinylalkyl glucosinolates into alkenyl glucosinolates (Fig. 2), and secondly, the production of hydroxypropyl glucosinolate. A third type of side chain modification, the addition of hydroxyl group to butenyl glucosinolate will be considered separately. A model of aliphatic glucosinolate biosynthesis is developed which is used to explain the diversity of aliphatic glucosinolates found in *A. thaliana* ecotypes.

Materials and methods

To investigate the conversion of methylsulphinylalkyl glucosinolates into alkenyl glucosinolates, a cross was made between the ecotype Limburg-5, which has pre-

Methionine

GSL-ELONG

PROPYLS

BUTYLS

Methylthiopropyl

Methylthiobutyl

Methylsulphinylpropyl

Methylsulphinylbutyl

GSL-OHP / \ *GSL-ALK*

GSL-ALK

Hydroxypropyl   Propenyl

Butenyl

GSL-OH

(2R)-2-hydroxy-3-butenyl
(2S)-2-hydroxy-3-butenyl

Side chain
elongation
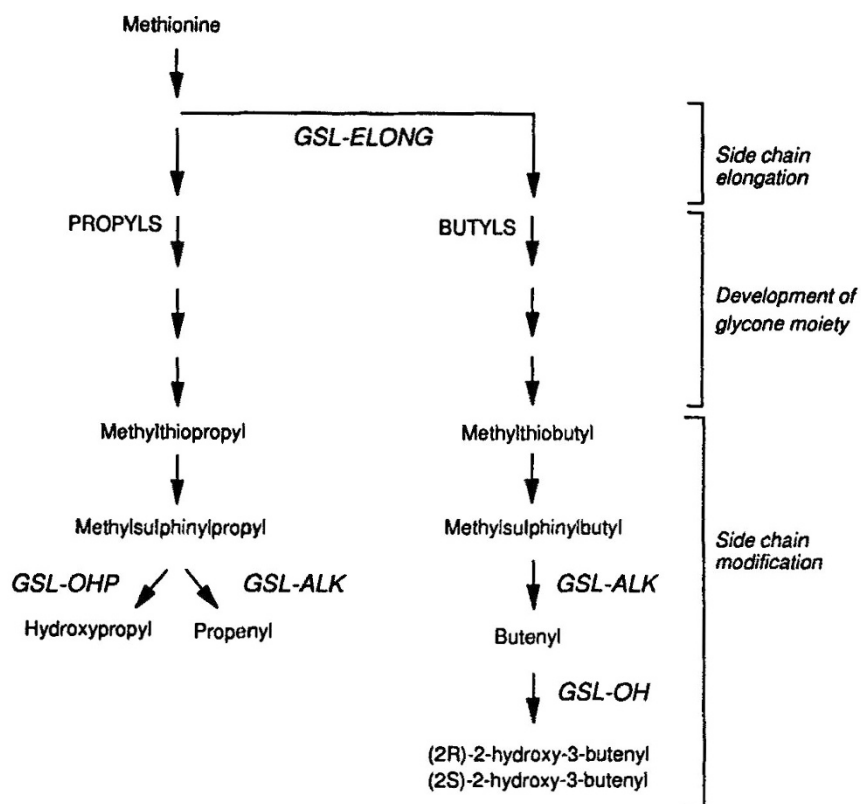
Development of
glycone moiety

Side chain
modification

Fig. 1 Genetic model of side chain elongation and modification of aliphatic glucosinolates in *Arabidopsis thaliana*.

dominantly methylsulphinylbutyl and methylsulphinyl-propyl glucosinolates and the inbred line H51 (a selection from the ecotype Stockholm) which has predominantly butenyl, hydroxybutenyl and propenyl glucosinolates (Fig. 2). The F_1 was selfed and F_2 and F_3 families generated, as described by Clarke & Dean (1994). All plants were grown under standard conditions as described previously (Magrath *et al.*, 1994). Glucosinolates were extracted from freeze-dried leaf tissue, converted to desulphoglucosinolates and analysed by HPLC as previously described (Magrath *et al.*, 1993). To investigate the production of hydroxy-propyl glucosinolate, the segregation of glucosinolate profiles in recombinant inbred (RI) lines from a cross between Landsberg *erecta*, which has predominantly hydroxypropyl glucosinolate, and Columbia, which lacks this glucosinolate (Fig. 3) was examined, as described previously (Magrath *et al.*, 1994). RFLP mapping in the cross between Limburg-5 and H51 was as described by Clarke & Dean (1994) and in the cross between Landsberg *erecta* and Columbia as described by Lister & Dean (1993). The glucosinolate profiles of F_1 hybrids between Columbia and H51, and between the ecotypes Kil-0 and Columbia were also examined.

Results

Cross 1: desaturation

The bulk tissue samples from the F_3 families had two distinct profiles which corresponded to the parental phenotypes (Fig. 2). Twenty-five families and the F_1 hybrid possessed predominantly butenyl, hydroxy-butenyl and propenyl glucosinolates (profile (b), Fig. 2) whereas 17 families possessed methylsulphinylbutyl and methylsulphinylpropyl glucosinolates (profile (a), Fig. 2), corresponding to the two parental phenotypes. The ratio is not significantly different from 3:1 $(0.05 > P > 0.01)$ suggesting the action of a dominant allele at a single locus $(GSL\text{-}ALK)$ which converts methylsulphinylalkyl glucosinolates into alkenyl glucosinolates. RFLP mapping places this locus between RFLP markers g3843 and w167 on chromosome 4 (Fig. 4).

Cross 2: hydroxylation of propyl glucosinolates

Four different glucosinolate profiles were found within the RI lines. Two of the profiles corresponded to the parental phenotypes (Fig. 3) and two were novel phenotypes, one of which contained methylsulphinyl-butyl and hydoxypropyl glucosinolates (Fig. 5a) and
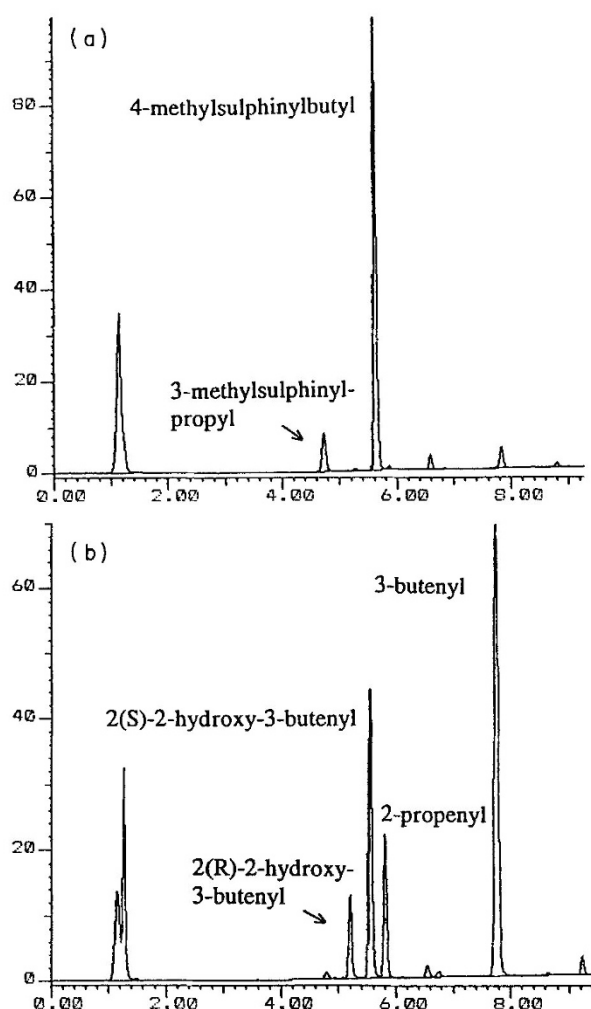
Fig. 2 Conversion of methylsulphinylalkyl glucosinolates to alkenyl glucosinolates in *Arabidopsis thaliana*. Limburg-5 has profile (a) and H51 profile (b). All recombinants corresponded to the two parental phenotypes.

glucosinolate fell to less than 10 per cent of the total aliphatic glucosinolate content (Fig. 5). RFLP mapping places this locus between RFLP markers w390 and m448 on chromosome 4, in a similar position to the *GSL-ALK* locus mapped in the first cross.

Hydroxypropyl glucosinolate may be derived from propenyl glucosinolate, in an analogous manner to which hydroxybutenyl glucosinolate is derived from butenyl glucosinolate. Hence if Columbia carried a 'silent' allele for hydroxylation, the locus mapped in cross 2 may regulate the desaturation of methylsulphinylpropyl glucosinolate, i.e. the same locus that was mapped in cross 1. To test this hypothesis, the glucosinolate profiles of two F_1 hybrids were examined, first that between Columbia and H51, and secondly between Columbia and the ecotype Kil-0, which only produced propenyl glucosinolate (Fig. 3). If Columbia carried a silent *GSL-OHP* allele, hydroxypropyl would be expected to occur in both of these F_1 hybrids. However, in the F_1 hybrids there was no evidence for this glucosinolate (Fig. 3). Therefore it appears that the gene mapped in cross 2 does regulate the hydroxylation of propyl glucosinolate. In F_1 hybrids between Columbia and Kil-0, hydroxybutenyl glucosinolate is found suggesting that one or both of the parents carry functional alleles for the hydroxylation of butenyl glucosinolate (Fig. 3). Hydroxybutenyl glucosinolate does not occur in either parent as both parents lack the precursor butenyl glucosinolate; Kil-0 as it lacks a functional allele at the *GSL-ELONG* locus and Columbia as it lacks a functional allele at the *GSL-ALK* locus (Fig. 1).

The functional *GSL-ALK* and *GSL-OHP* alleles may occur at two linked loci or may be different alleles at the same locus. A third allele at this locus (such as that which occurs in Columbia) would not result in any modification of methylsulphinylalkyl glucosinolates.

Discussion

The results indicate that two important side chain modifications of glucosinolates in *A. thaliana* are regulated by alleles on chromosome 4; firstly, the conversion of methylsulphinylalkyl to alkenyl glucosinolates and, secondly, the conversion of methylsulphinylpropyl glucosinolate to hydroxypropyl glucosinolate. It is likely that these alleles are at the same locus or are at two closely linked loci. A third allele, such as that found in Columbia, does not result in any modification of methylsulphinylalkyl glucosinolates. If the *GSL-ALK* gene were cloned it is possible that it could be used in antisense experiments in *Brassica*, either directly or by isolating the *Brassica* homologue. This should prevent the desaturation of methyl-
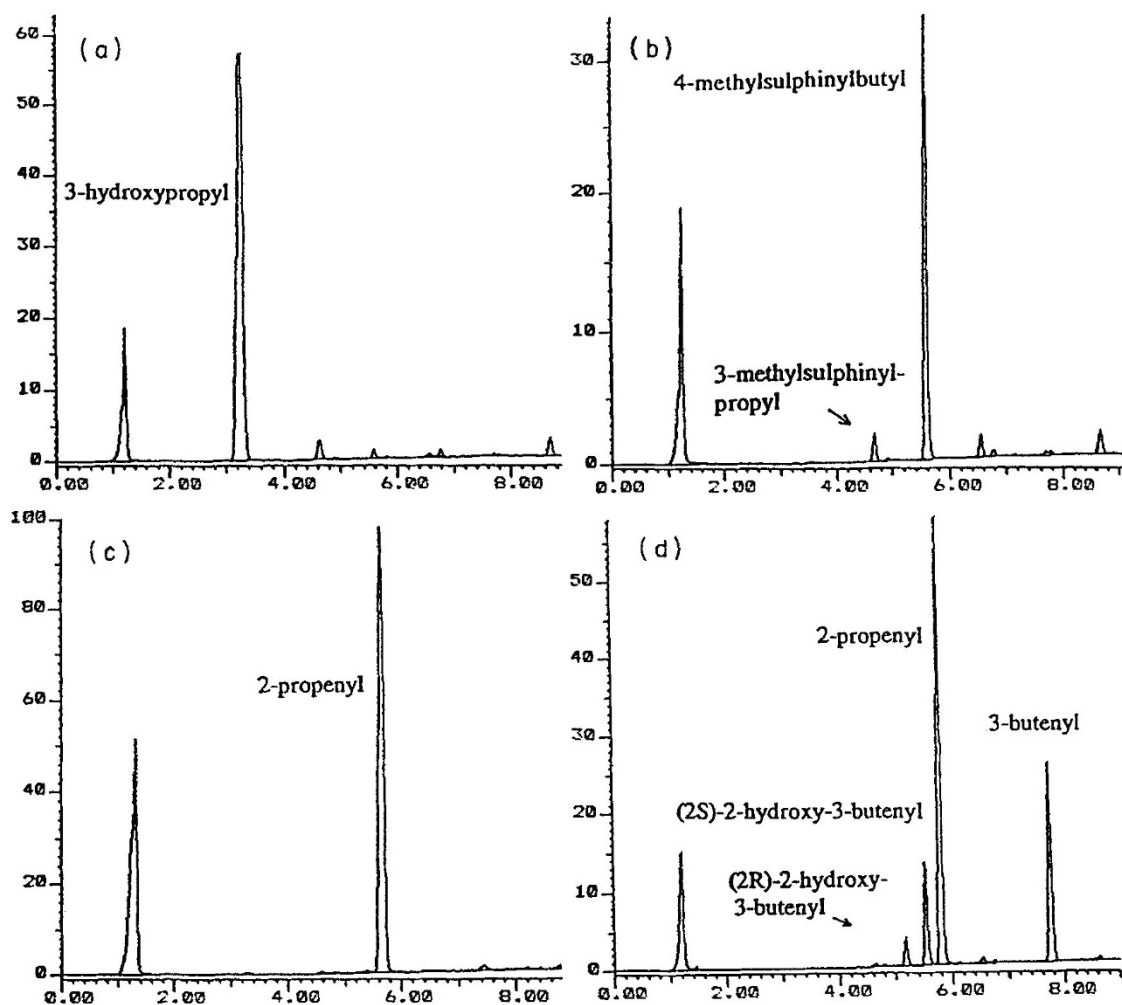
the other only methylsulphinylpropyl glucosinolate (Fig. 5b). The presence of methylsulphinylbutyl glucosinolate results from the inheritance of the Columbia allele at the *GSL-ELONG* locus described by Magrath *et al.* (1994). Fifty-three of the RI lines had significant amounts of hydroxypropyl glucosinolate whereas the remaining 47 lines had no detectable amounts of this glucosinolate. The ratio is not significantly different from a 1:1 $(P>0.05)$ suggesting the action of two alleles at a single locus (*GSL-OHP*). The proportion of hydroxypropyl glucosinolate depended on the presence of alleles at the *GSL-ELONG* locus (Magrath *et al.*, 1994); RI lines with the Landsberg *erecta* allele at this locus had 100 per cent hydroxypropyl glucosinolate whereas if the RI lines possessed the Columbia allele at this locus the proportion of hydroxypropyl

Fig. 3 Aliphatic glucosinolates of *Arabidopsis thaliana* Landsberg *erecta* (a), Columbia (b), Kil-0 (c) and the F_1 hybrid between Columbia and Kil-0 (d). The absence of hydroxypropyl glucosinolate in the F_1 hybrid suggests that Columbia does not carry a 'silent' allele at a putative *GSL-OHP* locus. Details of aliphatic glucosinolates in RI lines from a cross between Landsberg *erecta* and Columbia are given by Magrath *et al.* (1994).

sulphinylalkyl glucosinolates which would limit the production of hydroxyalkenyl glucosinolates, which are the major toxic components of rapeseed meal. This would provide an alternative approach to increasing the quality of rapeseed meal to that described by Parkin *et al.* (1994).

The variation in profiles observed in these ecotypes of *A. thaliana* can be explained by the simple genetic model shown in Table 1, in which the glucosinolates which are found in leaf tissue are determined by alleles at three loci: *GSL-ELONG* which regulates side chain elongation and is on chromosome 5 (Magrath *et al.*, 1994), *GSL-ALK/OHP* which regulates the conversion of methylsulphinylalkyl to alkenyl glucosinolates and the production of hydroxypropyl glucosinolate and is on chromosome 4 (this study), and a third

unmapped locus (unpublished data), designated *GSL-OH-Ar*, which regulates the hydroxylation of butenyl glucosinolate, analogous to the *GSL-OH-A* and *GSL-OH-C* loci found in *Brassica napus* (Parkin *et al.*, 1994). Combination of functional and null alleles at these three loci (Table 1) explains the variation in aliphatic glucosinolates found within the 63 ecotypes of *A. thaliana* described by Bano (1993). The absence of ecotypes with both propenyl and hydroxypropyl glucosinolates supports the hypothesis that the *GSL-ALK* and *GSL-OHP* alleles occur at the same locus. Both glucosinolates would only occur if the individual examined at this locus was heterozygous for these two alleles, which is unlikely considering the high degree of inbreeding which occurs in *A. thaliana*. A similar model explains the variation of aliphatic glucosinolates
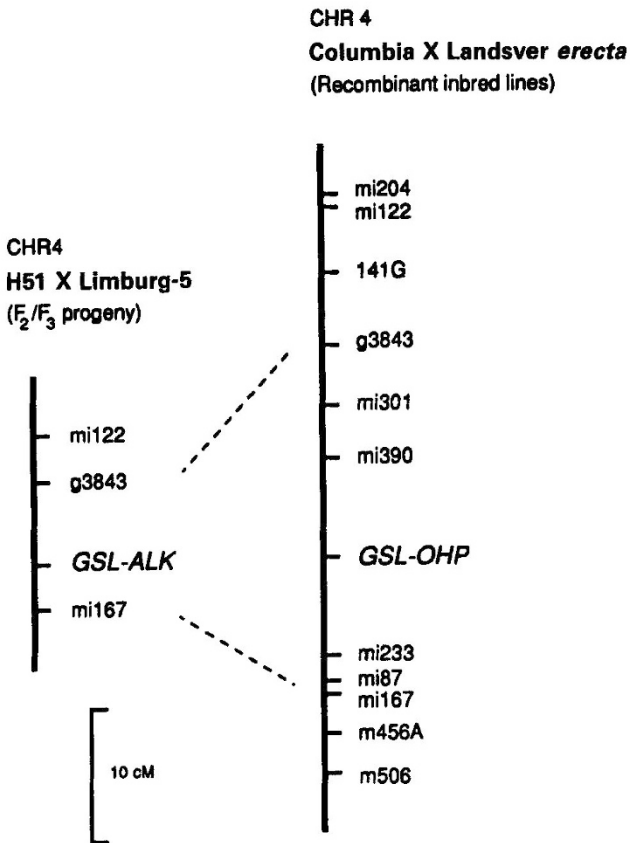
CHR4
H51 X Limburg-5
(F_2/F_3 progeny)

mi122
g3843
GSL-ALK
mi167

10 cM

CHR 4
Columbia X Landsver *erecta*
(Recombinant inbred lines)

mi204
mi122
141G
g3843
mi301
mi390
GSL-OHP
mi233
mi87
mi167
m456A
m506

Fig. 4 RFLP maps of the positions of the *GSL-ALK* and *GSL-OHP* loci of *Arabidopsis thaliana*, mapped in two separate crosses. Both loci map to the same interval.
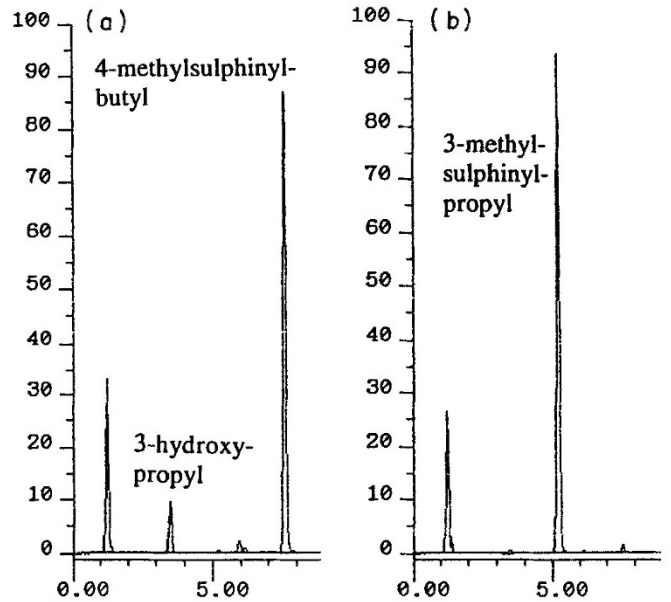

(a) 4-methylsulphinyl-butyl, 3-hydroxy-propyl

(b) 3-methyl-sulphinyl-propyl

Fig. 5 Novel aliphatic glucosinolate profiles in RI lines of *Arabidopsis thaliana* from a cross between Landsberg *erecta* and Columbia. Genotypes with profiles (a) combine the Columbia allele at the *GSL-ELONG* locus with the Landsberg *erecta* allele at the *GSL-OHP* locus whereas genotypes with profile (b) combine the Landsberg *erecta* allele at the *GSL-ELONG* locus with the Columbia allele at the *GSL-OHP* locus. Further information is given in Table 1.

Table 1 Genetic model for the phenotypic expression of aliphatic glucosinolates *Arabidopsis thaliana*. The *alk* and *ohp* alleles occur at the same locus (or at two loci which are closely linked) on chromosome 4. The model explains the variation in aliphatic glucosinolates reported to occur in *A. thaliana* ecotypes (Bano, 1993). Only homozygote genotypes are shown

Locus		Phenotype	Example
GSL-ELONG	*GSL-ALK/OHP*		
Alleles			
elong/elong	*alk/alk*	Butenyl (± hydroxylation), propenyl	H51 (Fig. 2b)
elong/elong	*ohp/ohp*	Methylsulphinylbutyl + hydroxypropyl	RI 194 (Fig. 5a)
elong/elong	*null/null*	Methylsulphinylbutyl + methylsulphinylpropyl	Limburg-5 (Fig. 2a) Columbia (Fig. 3b)
null/null	*alk/alk*	Propenyl	Kil-0 (Fig. 3c)
null/null	*ohp/ohp*	Hydroxypropyl	Landsberg *erecta* (Fig. 3a)
null/null	*null/null*	Methylsulphinylpropyl	RI 191 (Fig. 5b)

in *Brassica* (Magrath *et al.*, 1994; Parkin *et al.*, 1994; A. Giamoustaris and R. Mithen, unpublished data) and is likely to be applicable to the genetic regulation of aliphatic glucosinolates in other crucifers. This enables variation of aliphatic glucosinolates within and between wild crucifer populations to be interpreted in terms of the frequency of a small number of alleles.

Aliphatic glucosinolates mediate pest and pathogen interactions with crucifers. They decrease the palatability of leaf tissue to nonspecific herbivores such as slugs and birds, but increase the susceptibility of tissue to specialized insect pests such as flea beetles. It has been shown that both side chain length and modifications can affect the nature of these interactions. The differences in aliphatic glucosinolate content may be responsible for the variation in susceptibility of *A. thaliana* ecotypes to pests which is frequently observed when different ecotypes are grown under controlled conditions (personal observations). Likewise, natural populations of *A. thaliana*, *Brassica* and other crucifers may be exposed to different pest pressures in different environments which may lead to contrasting selection pressures on individuals with different aliphatic glucosinolate content. This may explain the intraspecific variation observed in aliphatic glucosinolate content both within and between populations. Fluctuating pest pressures, both in terms of the types (specialist vs. generalist) and frequency of pests, may result in contrasting selection pressures in different generations which would result in the maintenance of genetic diversity within populations.

Acknowledgements

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