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Phytochemical Correlates of Herbivory in a Community of Native and Naturalized Cruciferae

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Key Word Index—Plant-insect interactions; Cruciferae; glucosinolates; *Pieris napi macdunnoughii*; butterfly; oviposition; herbivory.

Abstract—Oviposition and larval feeding behaviors of the crucifer specialist *Pieris napi macdunnoughii* correlate with leaf glucosinolate profiles of plant species in a natural community. Profiles are species-specific in this group of eight Cruciferae, but particular glucosinolates are shared by subsets of the community. *Pieris* accepts two lethal naturalized weeds whose glucosinolate profiles resemble that of an indigenous foodplant. The results suggest that specific glucosinolates constitute insect behavioral cues which are only loosely linked evolutionarily to foodplant suitability, and further suggest that allelochemically similar community associates influence the coevolution of individual plant species with insect herbivores.

Introduction

Natural plant communities provide chemically and taxonomically diverse arenas in which plants and herbivorous insects interact and coevolve [1–6]. Many of these interactions are mediated in part by secondary plant metabolites with allelochemic effects [7–11]. The glucosinolates (mustard oil glucosides), for example, are a class of secondary compounds known to mediate interactions between Cruciferae (and some other glucosinolate-containing taxa) and their associated insect herbivores [12, 13] and fungal pathogens [14]. With few exceptions [15, 16], however, little is known about interactions in natural communities comprising several allelochemically similar plant species. While several predictions have been made about the community organization of chemically mediated plant–insect interactions [2, 5, 17–19], only limited data have been generated from studies of natural communities to test these often competing ideas.

In a comprehensive study of food-related behaviors of the crucifer specialist *Pieris napi macdunnoughii* Remington (Lepidoptera: Pieridae) in a montane area of Colorado, Chew [20, 21] documented differential growth rates and survival of larvae confronted with a community of six native and two naturalized species of Cruciferae. Table 1 summarizes the food-related behaviors of this butterfly as documented by Chew [20–22]. Four subsets of crucifers can be distinguished: (a) a preferred foodplant, *Descurainia richardsonii*, which supports significantly faster larval development than other species; (b) a group of four foodplants which support similar growth rates and among which *P. n. macdunnoughii* exhibits no preference, viz. *Arabis drummondii*,

TABLE 1. DIFFERENTIAL BEHAVIORAL AND GROWTH RESPONSES OF *PIERIS NAPI MACDUNNOUGHII* TO CRUCIFER LEAVES [20–22]

Crucifer species	*Pieris* responses		
	Lay eggs	Feed	Pupate
Descurainia richardsonii[i]	+[p]	+[p]	+[f]
Arabis drummondii[i]	+	+	+
Cardamine cordifolia[i]	+	+	+
Draba spectabilis[i]	+	+	+
Thlaspi montanum[i]	+	+	+
Erysimum asperum[i]	–	–	–
Chorispora tenella[n]	+	+	–
Thlaspi arvense[n]	+	+	–

Symbols: (+) acceptable; (–) not acceptable; (p) preferred; (f) fastest larval growth rate to pupation; (i) indigenous; (n) naturalized. This array includes all glucosinolate-producing species abundant in habitats of *P. n. macdunnoughii* near Gothic, Colorado.

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Cardamine cordifolia, Draba spectabilis, and *Thlaspi montanum*; (c) *Erysimum asperum,* which is unconditionally rejected by both adults and larvae; and (d) two naturalized weeds, *Chorispora tenella* and *Thlaspi arvense,* which are accepted by ovipositing females and feeding larvae but which fail to support larval development to pupation. The discrepancy between the behavioral and the growth responses of *P. n. macdunnoughii* to the naturalized species suggests that the *Pieris* do not recognize these two as lethal. Chew [22] also found that ovipositing butterflies exhibit a slight preference for *D. richardsonii,* the foodplant which was also consistently preferred by larvae. These results raise the question, do glucosinolate differences in this array of potential foodplants account for the established differences in food-related behaviors by this specialist herbivore?

We report here results from paper- and gas-chromatographic analyses of the leaf glucosinolate composition in this array of potential foodplants confronting *P. n. macdunnoughii* in montane Colorado. Profiles of leaf glucosinolates (defined as kinds and proportions of constituents) are species-specific in this community of eight Cruciferae, and pierid behavioral responses to these crucifers correlate with particular aspects of the profiles. Two naturalized weeds, *Ch. tenella* and *Th. arvense,* which are fed upon by larvae but are lethal, produce a presumptive attractant, allylglucosinolate, which is otherwise present only in the preferred foodplant, *D. richardsonii.* The chemical data are the first published results for a diverse community of crucifers, and provide the first results for intra-populational sampling of leaf glucosinolates in non-cultivated taxa. The results for two of the species, *A. drummondii* and *C. cordifolia,* provide suggestive evidence for glucosinolate polymorphism within natural populations. From the observations and correlations, we suggest some of the plausible outcomes of the coevolutionary interaction between foodplants and their adapted specialist herbivores.

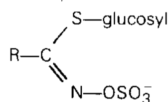
Results

Table 2 presents the results of our paper-chromatographic (PC) and gas-chromatato-

TABLE 2. LEAF GLUCOSINOLATE PROFILES OF THE EIGHT GOTHIC CRUCIFERAE, DETERMINED BY PC AND GC OF HYDROLYSIS PRODUCTS.

		Glucosinolate																					
Plant species		1. Isopropyl-	2. 2 Hydroxy-1-methylethyl-	3. 2-Butyl-	4. 1-(Hydroxymethyl)propyl	5. Isobutyl-	6. 2-Hydroxy-2 methylpropyl-	7. Allyl-	8. 3-Butenyl-	9. 4 Pentenyl-	10. 3-Methylthiopropyl-	11. 3-Methylsulfinylpropyl	12. 4-Methylsulfonylbutyl-	13. 5-Methylthiopentyl-	14. 5-Methylsulfinylpentyl-	15. 6-Methylthiohexyl	16. 6-Methylsulfinylhexyl-	17. 6-Methylsulfonylhexyl-	18. 8-Methylsulfinyloctyl-	19. Benzyl	20. 2-Phenylethyl-	21. Unknown I	22. Unknown II
Descurainia richardsonii	(17)	+		+		+		+	+ +	+			+	+						+			
Arabis drummondii	(31)	+	+	+ +		+									+	+	+ +	+		+			
Cardamine cordifolia	(17)	+ +	+	+ +	+	+ +	+													+	+		
Draba spectabilis	(5)	+ +		+															+ +				
Thlaspi montanum	(4)	+		+																		+ +	+
Erysimum asperum	(2)											+ +	+ +										
Chorispora tenella	(1)							+ +			+	+											
Thlaspi arvense	(3)							+ +				+								+			

Semisystematic chemical names of glucosinolate R-groups are listed:



Quantitatively major compounds (+ +) are distinguished from minor or trace components (+); see text for criteria. Numbers in parentheses following species names are the number of samples analysed. Glucosinolates 1–2, 3–4, 5–6, 7–18, and 19–20 appear to be biosynthetically related [23, 24]. Unknown I, the major leaf glucosinolate in the four samples of *Thlaspi montanum,* has tentatively been identified on the basis of PC mobility values [27] as (*p*)-rhamnopyranosyloxybenzylglucosinolate.

graphic (GC) analyses for leaf glucosinolates for the eight Cruciferae confronting *P. n. macdunnoughii* in montane meadows around Gothic, Colorado. Quantitatively major compounds are distinguished from minor ones in Table 2, based on color intensity of spots by PC and on an arbitrarily chosen value of 10% peak area by GC. The range of compounds detected for a species, both in number of entities and in presumed biosynthetic diversity [23, 24], is similar to that found for leaf glucosinolates of cabbage cultivars [25]. The glucosinolate profiles were consistent among samples of a species (see Table 3 for *D. richardsonii*) with two exceptions, although minor or trace compounds were occasionally absent from samples comprising only a few leaves.

Only in *A. drummondii* and *C. cordifolia* was significant variation detected in the major glucosinolates comprising the profile. Tables 4 and 5 present results on the potential glucosinolate polymorphisms found in the Gothic populations of these two species. In *A. drummondii* (Table 4) most plants contained large proportions of 2-butylglucosinolate and smaller amounts of isopropylglucosinolate, but in two individuals the relative amounts were reversed. In two other individuals a cyclic oxazolidinethione was detected on enzymatic hydrolysis; this has tentatively been identified from PC mobility values as 4-methyloxazolidine-2-thione, deriving from the 2-hydroxylated analogue of isopropylglucosinolate [26]. The

TABLE 3. LEAF GLUCOSINOLATE COMPOSITION OF 14 INDIVIDUAL AND 3 BULK SAMPLES OF GOTHIC *DESCURAINIA RICHARDSONII*

Descurainia richardsonii	Glucosinolate								
	Isopropyl-	2-Butyl-	Isobutyl-	Allyl-	3-Butenyl-	4-Pentenyl-	5-Methylthiopentyl-	4-Methylsulfonylbutyl-	Benzyl-
Collection	% by GC								
Chew *s.n.* 1977			t	t	99				
R397	t	t		3	95	1			t
R408-1		t			99				t
R408-2		t		3	97	t			t
R408-3				3	97	t			
R408-4		t	t	3	97	t			t
R408-5		t		t	99				t
R408-6	t	t		3	97	t			
R408-7		t	9	2	89	t			t
R408-8		t		3	97	t			t
R408-9				4	96	t			t
R408-10	t	1		3	96	t			t
R408-11		t		4	96	t			t
R408-12				4	96	t			
	Analysed by PC								
C 23				+	++				
C 32/33*				+	++		+	+	
Chew *s.n.* 1974				+	++		+	+	

Determined by GC of volatile isothiocyanates for individual samples (peak area percentage determined by triangulation) and by PC of hydrolysis products for bulk samples (relative amounts determined by color intensity and size of spots). No cyclic oxazolidinethiones were detected in any of the samples. t = trace amount (less than 1% of total peak area); (+ +) = major component; (+) = minor or trace component; * = sample split to test for hydrolysis products from endogenous leaf myrosinase vs exogenous (*Sinapis alba*) [27] myrosinase activity, with no differences detected.

TABLE 4. LEAF GLUCOSINOLATE COMPOSITION OF 25 INDIVIDUAL AND 6 BULK SAMPLES OF GOTHIC *ARABIS DRUMMONDII*

Arabis drummondii	Glucosinolate								
	Isopropyl-	2-Hydroxy-1-methylethyl-	2-Butyl-	Isobutyl-	5-Methylsulfinylpentyl-	6-Methylthiohexyl-	6-Methylsulfinylhexyl-	6-Methylsulfonylhexyl-	Benzyl-
Collection	% by GC								
R402-1	99		1						t
R402-2	98		2						t
R402-3	4		96						
R402-4	3		97						t
R402-5	5		95						t
R402-6	5		95						t
R402-7	4		96						t
R402-8	5		92	2					t
R402-9	5		92	2					1
R402-10	4		96						t
R402-11	3		94	2					t
R402-12	4		92	3					
R402-13		++							t
R402-14	3		97						t
R402-15	4		96						
R402-16	4		96						
R402-17	3	+	97						t
R402-18	5		95						
R402-19	4		96						t
R402-20	4		96						
R402-21	5		93	2					t
R402-22	4		96						t
R402-23	5		95						
R402-24	3		97						
R402-25	2		98	t					
	Analysed by PC								
R267	+		++		+		++	+	
Chew *s.n.* 1974	+		++		+		+		
C 2	+	+	++				+		
C 21		+					++		
C 31		+					++		
C 36/37*	+	+	++		+	+	+		

Determined by GC of volatile isothiocyanates for individual samples (peak area percentage determined by triangulation) and by PC of hydrolysis products for bulk samples (relative amounts determined by color intensity and size of spots). Individual samples further tested for cyclic oxazolidinethiones by PC of extracts originally prepared for GC [27]. t = trace amounts (less than 1% of total peak area); (+ +) = major component; (+) = minor or trace component; * = sample split to test for hydrolysis products from endogenous leaf myrosinase vs exogenous [27] myrosinase activity, with no differences detected.

cyclic compound was found to the exclusion of the non-hydroxylated analogue in two bulk samples of the species. In *C. cordifolia* (Table 5) nine of 13 individual plants gave positive results in tests for cyclic oxazolidinethiones; these were tentatively identified from PC mobility values [27] as 2-hydroxylated analogues of the principal glucosinilates: isopropyl, 2-butyl, and isobutyl. Whether the variation detected in *A. drummondii* and *C. cordifolia* constitutes genetic polymorphism or results from environmental or developmental effects is unknown and is under further study.

Two features of the results presented in Table 2 deserve emphasis: (a) the species-specific leaf glucosinolate profiles, and (b) the shared occurrence of particular glucosinolates.

(a) Each species in this community produces a distinctive leaf glucosinolate profile, and thus possesses a distinctive 'chemical halo' [28]. In some cases single major glucosinolates uniquely distinguish particular species, for example 3-butenylglucosinolate in *D. richardsonii*; in other instances, minor constituents or distinctive proportions of several components serve to characterize the species. Taxonomically diagnostic glucosinolate profiles have been demonstrated in other Cruciferae. For example, cultivar-specific arrays of leaf glucosinolates have been documented in cabbages [25], and distinctive seed glucosinolate profiles have been documented in the genera *Thelypodium* [29] and *Cakile* [30–32].

A review of the literature has revealed data on glucosinolate composition in only two of the species under study here. Our findings agree with the report by Gmelin and Virtanen [33] of allylglucosinolate in leaves of *Th. arvense* (cf. [13]). In addition, we detected small amounts of benzylglucosinolate and also small amounts of 3-methylsulfinylpropylglucosinolate; the latter appears to be biosynthetically related to the allyl constituent [34, 35]. We have found this same array of glucosinolates in collections of *Th. arvense* from New Haven, Connecticut (Rodman, J. E., unpublished data). For *A. drummondii* Schraudolf [36] reported 3-indolylmethylglucosinolate in etiolated seedlings. Indolic glucosinolates are common in vegetative tissues of Cruciferae, but usually in concentrations far less than other glucosinolates [37]. We obtained only weakly positive results for indolic constituents from a few samples of all the taxa we examined.

(b) Particular glucosinolates are shared among taxonomically distinct entities in this community. With reference to shared constituents, three subsets of this crucifer assemblage can be distinguished and correlated with the food-related behaviors of *P. n. macdunnoughii* (see Table 1). The first subset, characterized by allylglucosinolate, includes *D. richardsonii* and the two naturalized and lethal weeds, *Ch. tenella* and *Th. arvense*. The second, characterized by the chemically homologous [26] isopropyl and 2-butyl glucosinolates and/or their hydroxy analogues, comprises the four species toward which *P. n. macdunnoughii* shows similar responses, viz. *A. drummondii, C. cordifolia, D. spectabilis,* and *Th. montanum*. The third subset is *E. asperum* in which the major leaf glucosinolate is the 3-methylsulfinylpropyl compound. Thus the preferred foodplant and the lethal weeds

TABLE 5. LEAF GLUCOSINOLATE COMPOSITION OF 13 INDIVIDUAL AND 4 BULK SAMPLES OF GOTHIC *CARDAMINE CORDIFOLIA*

Cardamine cordifolia	Glucosinolate							
Collection	Isopropyl-	2-Hydroxy-1-methylethyl-	2-Butyl-	1-(Hydroxymethyl)propyl-	Isobutyl-	2-Hydroxy-2-methylpropyl-	Benzyl-	2-Phenylethyl-
	% by GC							
Chew *s.n.* 1977	15		64		17		4	
R409-1	3	+	78	+	17		2	t
R409-2	6		78		12	+	4	t
R409-3	15		61		16	+	3	4
R409-4	7		78	+	11		4	t
R409-5	42		46	+	12			t
R409-6	t		43	+	32		17	8
R409-7	t		86		14			t
R409-8	3		71	+	16			10
R409-9	51		40	+	6		t	3
R409-10	6		76		12		t	5
R409-11	4	+	56	+	17		23	t
R409-12	29		29		42			
	Analysed by PC							
R255	+	+	+ +	+ +				
Chew *s.n.* 1974	+ +		+					
C 25	+		+ +					
C 45/46*	+		+ +					+

Determined by GC of volatile isothiocyanates for individual samples (peak area percentage determined by triangulation) and by PC of hydrolysis products for bulk samples (relative amounts determined by color intensity and size of spots). Individual samples tested for cyclic oxazolidinethiones by PC of extracts originally prepared for GC [27]. t = trace amounts (less than 1% of total peak area); (+ +) = major component; (+) = minor or trace component; * = sample split to test for hydrolysis products from endogenous leaf myrosinase vs exogenous [27] myrosinase activity, with no differences detected.

share the allyl compound while the other acceptable crucifers and the rejected *Erysimum* constitute chemically distinct groups.

Discussion

Gustatory stimuli play a major role in foodplant selection by lepidopteran larvae [12, 38–40], and specifically for caterpillars of the genus *Pieris,* the glucosinolates are an important class of chemical cues [12, 41–44]. Furthermore, differential feeding stimulation by various glucosinolates, tested under laboratory conditions, has been demonstrated in *Pieris brassicae* [41, 45] and in other crucifer specialists [41, 46, 47]. Schoonhoven [43, 44] has demonstrated that specific contact chemoreceptors of *P. brassicae* larvae respond differentially to various glucosinolates, and that it is the glucoside rather than the aglucone to which insect receptors are more sensitive. Similarly, ovipositing *Pieris* appear to utilize glucosinolate cues in selecting suitable plants [48]. Ma and Schoonhoven [49] have described tarsal contact chemoreceptors in *P. brassicae* which are specifically sensitive to glucosinolates. These compounds have also been shown to be oviposition stimulants for several other crucifer specialists [50–52]. It is likely that ovipositing females respond differentially to different glucosinolates and/or isothiocyanates [53]. This discriminatory faculty provides a basis for understanding insect preferences among chemically related but distinctive potential hostplants.

Given the distinctive leaf glucosinolate profiles for these crucifers and the observed correlations with food-related behaviors of *P. n. macdunnoughii*, we suggest that adults and larvae of this butterfly discriminate among the crucifers at least partly on the basis of particular leaf glucosinolates. Since females oviposit on and larvae feed upon two naturalized weeds, even though these are lethal, we conclude that allylglucosinolate in these two species and in *D. richardsonii* is an important attractant for this insect. (It is possible that *P. n. macdunnoughii* does not behaviorally discriminate allylglucosinolate from 3-butenylglucosinolate, the latter major compound in *D. richardsonii* differing only in a single methylene group; this does not affect the logic of our argument.) Allylglucosinolate, in particular, has been shown to be a feeding and ovipositing stimulant for *P. brassicae* and *P. rapae* [12, 44, 48, 49]. The four foodplants among which *P. n. macdunnoughii* exhibits no preference (*A. drummondii, C. cordifolia, D. spectabilis, Th. montanum*) presumably also provide feeding and ovipositing attractants (probably one or some combination of the first four compounds listed in Table 2), but these may not be as effective as allylglucosinolate.

Alternatively, but less parsimoniously as explanation, the foliage glucosinolates of the Gothic crucifers may serve only as a general class of behavioral attractants to *P. n. macdunnoughii,* with other, as yet unidentified, constituents modifying insect behavior to produce the differential responses observed (cf. [54]). In this case, compounds other than specific glucosinolates must be invoked to explain the feeding and ovipositing preferences associated with *D. richardsonii.* Furthermore, factors other than glucosinolates must then be invoked to explain the avoidance of *E. asperum* by this butterfly. The proximate cause of this non-exploitation may be either lack of appropriate attractants (i.e. crypsis) or presence of chemical repellents (i.e. deterrency). Crypsis seems an unlikely explanation since *Erysimum* does produce glucosinolates (although, alone among this array of crucifers, *Erysimum* produces glucosinolates whose isothiocyanates are not volatile). Indeed, its major constituent (3-methylsulfinylpropylglucosinolate) has been shown to be a weak feeding stimulant for *P. brassicae* larvae [45]. Repellency seems a more likely explanation [55, 56], but the chemical or physical nature of the repellent(s) remains unknown. Feeny [13] has argued that cardenolides, which are present in European *Erysimum* species, may function as insect feeding deterrents, and Nielsen [54] has provided experimental evidence that cardenolides deter feeding by certain *Phyllotreta* flea beetles which specialize on Cruciferae. However, Usher [57] has recently demonstrated that the cardenolide k-strophanthin has no toxic or repellent effect on larvae of *Pieris rapae.*

One alternative deterrent in *E. asperum* might be the major glucosinolate itself. While glucosinolates vary in their effectiveness as feeding stimulants, repellency toward adapted herbivores has been demonstrated only for the allyl compound tested at high concentrations [47]. Blau *et al.* [58] found no effects on fifth-instar larval growth rates of *Pieris rapae* fed collard leaves boosted with allylglucosinolate, but Marsh and Rothschild [59] reported very high pupal mortality in the species, following larval rearing on cabbage leaves boosted with

0.25 to 1% solutions of this compound. In experimental tests with larvae of the crucifer specialist *Plutella maculipennis,* two glucosinolates which weakly stimulated feeding (3-butenyl and 2-phenylethyl glucosinolates) were found to be toxic to these larvae [46]. If these glucosinolates were persistent components of the environment of this insect, one would expect selection over time for recognition and avoidance of them. One alternative hypothesis then (to the cardenolide one) is the repellency, and possible toxicity, of 3-methylsulfinylpropylglucosinolate for *P. n. macdunnoughii.* We are encouraged toward this speculation by the possibility, intriguing for its ecological parsimony, that the lethality of *Ch. tenella* and *Th. arvense* may be due to the presence of small but consistent amounts of this same compound, otherwise masked by the more abundant allylglucosinolate in leaves of these plants. Feeny [13] has argued that *Th. arvense* is likely to be toxic to crucifer specialists because leaves of this plant yield allyl thiocyanate upon hydrolysis of their allylglucosinolate rather than the more usual allyl isothiocyanate [33]. However, our preliminary tests using endogenous enzymes in Gothic *Th. arvense* leaves revealed allyl isothiocyanate as the major hydrolysis product. There may exist geographic variation within this species in the enzymatic capacity to form thiocyanates, analogous to organ variation within plants of *Lepidium sativum* in the capacity for thiocyanate formation [60].

Larval mortality on the introduced weeds *Ch. tenella* and *Th. arvense* emphasizes that glucosinolates are not necessarily intrinsic indicators of foodplant quality. Rather, individual glucosinolates become specific indicators for *Pieris* over evolutionary time, because each is perceived to be associated with foodplants of a particular quality. Selective larval mortality on nutritionally inadequate or toxic plants can be expected to reinforce discriminatory preferences for acceptable foodplants, with concomitant selection for sensory recognition of specific glucosinolates associated with these plants. We predict that in the Gothic community ovipositing *Pieris* will eventually discriminate against *Ch. tenella* and *Th. arvense,* although not on the basis of major glucosinolates shared with native foodplant species. Alternatively, because ovipositing *P. n. macdunnoughii* currently exposes larvae to these toxic weeds, selection will favor any physiological change which permits larvae to utilize these plants successfully. The partial discrimination against *Th. arvense* shown by ovipositing females of another indigenous pierine in this community, *Pieris occidentalis* Reakirt [22], whose larvae also die after feeding on this weed [21], suggests that behavioral recognition and avoidance may constitute the more likely initial response.

A plant population which is associated with a particular chemical signal may change its vulnerability to insect attack by changing its allelochemic profile [61], thus altering the insects' perception of the quality of the plant (cf. [62]). This evolutionary response to herbivore attack is likely to be canalized by existing plant biosynthetic pathways [63–65]. For the glucosinolate-producing Cruciferae, modification either of biosynthesis or of enzymatic hydrolysis of glucosinolates represents two potential foci of allelochemic change. With reference to biosynthetic modifications, it is significant that the species-specific glucosinolate profiles of the crucifers reported here comprise clusters of biosynthetically related compounds. Three patterns reflect presumed biosynthetic relationships [23, 24]: (a) hydroxylation, which accounts for the intrapopulational polymorphisms encountered in *A. drummondii* and *C. cordifolia*; (b) homologization or chain extension, for example, the series of alkenyl glucosinolates in *D. richardsonii* (compounds 7–9 in Table 2); and (c) oxidation of the *R*-moiety sulfur atom, for example the series of compounds 15–17 (Table 2) in *A. drummondii.* Alternatively, crucifer response to insect attack over evolutionary time may involve change in the products of glucosinolate hydrolysis, presumably through modification of plant hydrolase activity [60]. Different classes of hydrolysis products, for example, isothiocyanates and thiocyanates, are potentially distinguishable from each other and from glucosinolates by crucifer specialists [13].

Our findings of distinctive glucosinolate profiles in these eight sympatric Cruciferae and, preliminarily, of intra-populational polymorphism in two of them are consistent with, though not a direct test of, the prediction that herbivore pressure leads to divergence in plant chemical defenses [2, 5, 6, 10]. This divergence, furthermore, should be canalized by the plants' existing biosynthetic capabilities, leading to the production of arrays of biosynthetically related compounds as found in the crucifers reported here. Experimental tests of

some of the ideas proposed here (for example, the repellency and/or toxicity of 3-methylsulfinylpropylglucosinolate to *P. n. macdunnoughii*) will help to demonstrate causal connections where we now have only correlations. Further comparative studies of natural communities, particularly those differing in degree of herbivore pressure, are required in order to elucidate the processes of allelochemic divergence.

Experimental

The plants analysed in this study are listed in Table 1 with nomenclature following Weber [66], or Harrington [67] for *Draba spectabilis*; voucher specimens are deposited in the Yale University Herbarium. All samples were collected from Chew's study sites [20–22] along the Copper Creek drainage or from adjacent meadows in the Gothic area. These plant species constitute the potential foodplants available to *P. n. macdunnoughii* in montane meadows (*ca.* 2750–3450 m) around the Rocky Mountain Biological Laboratory at Gothic, Gunnison County, Colorado, the site of our field studies. This array of Cruciferae does not exhaust the entire 'universe' of potential foodplants for the insect in this part of its range [68], but we selected all the abundant species as revealed by intensive field observations over several seasons (cf. [69, 70]).

PC and GC analyses were performed on rosette and/or cauline leaves on which female butterflies oviposit and caterpillars feed. The various samples were collected over five field seasons (1974–1978) although the intra-populational samples of *A. drummondii* (collection R402 in Table 4), *C. cordifolia* (R409 in Table 5), and *D. richardsonii* (R397 and R408 in Table 3) were all made in 1978 (by J.E.R.). These intra-populational samples were made from individuals judged to be at a similar developmental stage, i.e. recently bolted (flowering) plants. Leaves from one plant (individual sample) or from two to five plants (bulk sample) were collected in the field in 70% MeOH, boiled soon afterward in the same solvent to denature endogenous enzymes, and the extract stored for up to 3 months before analysis. Fresh plants of *C. cordifolia* (Table 5: Chew *s.n.* 1977) and *D. richardsonii* (Table 3: Chew *s.n.* 1977), potted in soil and air-shipped to New Haven, were analysed by PC and GC without an intervening storage; the results were fully consistent with extracts from plants stored several months.

The PC and GC methods used for compound separation and identification have been described [30, 31]; the GC column, equipment, and protocol described in [31] were used, and the modification described in [29] for handling leaf material for PC was adopted. PC facilitates identification of individual glucosinolates based on analysis of the corresponding thiourea derivatives of the enzymatically released isothiocyanates and on analysis of the cyclic oxazolidinethiones generated spontaneously from 2-hydroxylated isothiocyanates [27]. PC also provides an estimate of relative proportions of constituents, determined by color intensity and size of spots. GC corroborates compound identification for volatile isothiocyanates and provides quantitative estimates of relative amounts as reflected by relative peak areas on gas chromatograms, measured by triangulation. GC also provided estimates of total glucosinolate content (cf. [27]) for single-plant samples of *C. cordifolia* and *D. richardsonii*, viz. 0.34 mg/g fr. wt and 0.10 mg/g, respectively.

For all taxa except *Ch. tenella*, one sample was split and the two portions were hydrolysed either with exogenous myrosinase as in most of the analyses [27] or with an aqueous extract of fresh leaves prepared at the time of collection; in all cases, similar hydrolysis products were obtained. These results indicate that the hydrolysis products detected in our laboratory analyses duplicate those that herbivores are likely to encounter when feeding on leaves in nature.

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