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Patterns of resource use by milkweed insects in Sinai

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Abstract

Plant morphology and defensive chemistry are related to the insect community of herbivores on *Gomphocarpus sinaicus* (Boiss.) (Apocynaceae) in Sinai (Egypt). There appears to be significant variation among individual plants in the components of their chemical defences. The different components of the community respond differently to plant characters; plant defence appears to be an important determinant of the relative abundances of the insect species. The data showed an indications of different relationships of the insect herbivores to levels of chemical defences, especially aphids. While weevil and bug densities covaried, those of aphids varied more independently, and possibly inversely. The community is compared with the much better known North American herbivore community on plants of the sister-genus *Asclepias*.

Keywords: *Asclepias*, *Gomphocarpus*, aposematism, cardenolides, coevolution, insect-plant interactions, specialisation

Introduction

Host-plant defensive chemistry is known to mediate food choice in many herbivorous insects through its effects on food quality limiting growth, survival and fecundity (Ode 2006), which are usually but not always negative (eg del Campo *et al* 2005). On the other hand, many herbivores use these defensive chemicals in their own defence, and this usage also has a powerful influence on host choice (eg Denno *et al* 1990). The relative importance of food quality versus the opportunity to generate 'enemy free space' is an interesting and important issue (Strauss & Zangerl 2002), since only a proportion of herbivores evolves the ability to excrete, modify (eg by detoxification) or to sequester the plant defensive chemicals they encounter, presumably because of the entailed costs (Després *et al* 2007). A further influence on food choice is the community context, since interactions among animal species can be mediated through a common host-plant (eg Soler *et al* 2007) via a variety of mechanisms, such as trait-mediated indirect effects (Abrams *et al* 1996). An obvious example is changes in plant resistance induced by prior attack. Such interactions can also have evolutionary consequences: for example, beak morphology in crossbill birds is under selection from squirrels causing changes in pine cone structure (Edelaar & Benkman 2006, Siepielski & Benkman 2007).

Here we examine the correlation structure of herbivore assemblages associated with a chemically well-defended milkweed species *Gomphocarpus sinaicus* (=*Asclepias sinaica*) for evidence of host-plant selection influenced by defensive chemistry, the ability to detoxify or to sequester, and possible host-plant mediated competition. Like all milkweeds (Wyatt & Broyles 1994), *Gomphocarpus sinaicus* is defended by cardiac glycosides (cardenolides) (Elaskary *et al* 1995a), known to be toxic to many insects (eg Malcolm 1990, 1995), and also contains flavonoids that may also be toxins (El Batran *et al* 2005). In southern Sinai, Egypt, *Gomphocarpus* is host to a relatively simple community of insect herbivores (a lygaeid bug, an aphid and a weevil) and their predators (syrphid larvae, coccinellids and several bird species). The lygaeid and the aphid are both aposematic and known to sequester host-plant toxins. The weevil is a specialist, the aphid a moderate specialist, whereas the lygaeid is a generalist herbivore. Cardenolide concentrations and latex production are now known to be at least partly

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under genetic control in *Asclepias syriaca*, with heritabilities of about 0.20 (Agrawal 2004a, Mooney & Agrawal 2008). We ask whether plant morphology and plant chemistry, in particular the concentrations of cardiac glycoside toxins, are associated with host-plant use by these herbivores and the coccinellid predators. We show that host-plant use is probably influenced by all these factors.

Materials & Methods

Our study was carried out between 2000 and 2005 in Wadi Arbaein (28° 32’ 35.65” N, 33° 57’ 28.5” E, 1620 m altitude), St Katherine Protectorate, Sinai, Egypt (see Zalat & Gilbert 1998, 2008 for further habitat details). The perennial shrub *Gomphocarpus sinaicus* (Boiss.) (Apocynaceae: Asclepiadoideae) is the only Middle Eastern member of the mainly African genus *Gomphocarpus*, the sister-group (Goyder & Nicholas 2001) to the well-known New World genus *Asclepias* (in which it is sometimes placed, eg Boulos 2000). It occurs in Sinai, the Hedjaz of Saudia Arabia and the mountains of adjacent countries. It is a relatively common plant in the bottom of Wadi Arbaein in the St Katherine Protectorate, patchily distributed along the wadi floor at low densities (as is every other plant in this hyperarid environment). It occurs in some wadis but not others, and is largely absent from the highest wadis. Like all asclepiads, *Gomphocarpus sinaicus* flowers have a complex structure and pollination (Wyatt & Broyles 1994). They flower twice during the year, in March-May and again in August-September (Elbanna 2004), and the resulting follicles are very variable in size. Also like other asclepiads (e.g. Seiber *et al* 1982), *Gomphocarpus sinaicus* contains cardenolide glycosides (Elaskary *et al* 1995a, b; Abdel-Azim *et al* 1996; Abdel-Azim 1998) and is therefore almost certainly toxic to many insects. The main cardenolide glycoside in all plant parts is reported to be 5, 6-dehydrocalotropin and there are said to be no large differences in the concentration of 5, 6-dehydrocalotropin among the plant tissues, although seeds and roots have lower concentrations (Elaskary *et al* 1995b) (but see our results).

The milkweed bug *Spilostethus pandurus* Scopoli (Hemiptera: Lygaeidae) is a generalist aposematic seed predator, mainly associated with *Gomphocarpus sinaicus* in Sinai, but at low densities (Elbanna 2004). Individuals are active mainly from April to September, with a few still evident on plants into the winter; there appears to be two generations per year (or part of the population manages two generations), with nymphs dominating by July and appearing again in October: both adults and nymphs over-winter (Elbanna 2004). Adults are diurnal and crepuscular, active during the morning (peaking at 1000 h) and evening (peaking 1900 h), presumably to avoid high daytime temperatures (max. 35-38 °C) since winter-active individual are unimodal, peaking at 1100 h. Siblings stay together on a plant and aggregate at night and over the winter. Adults are capable of flying between plants, but rarely do so, and appear to return to the same ‘home’ plant to spend the night. Importantly, *Spilostethus pandurus* is able to sequester cardiac glycosides from *Gomphocarpus sinaicus* for its own defence (Elbanna 2004; cf. von Euw *et al* 1971). *Gomphocarpus* seeds contain lower levels of glycosides, and therefore this herbivore generally encounters lower levels than the other insect herbivores in the system, although it also feeds from stems and the follicle wall where glycoside levels are much higher.

The oleander aphid *Aphis nerii* Boyer de Fonscolombe (Homoptera: Aphididae) is a bright-yellow aposematic insect specialising on a few genera of Apocynaceae (Martel & Malcolm 2004): in Sinai it infests various plant parts (young leaves, growing shoots and follicles), and possibly also occurs on *Verbascum* (Scrophulariaceae). It is known to be capable of sequestering cardenolides for defence against predators (Malcolm 1990).

The milkweed weevil *Paramecops sinaitus* Pic (Coleoptera: Curculionidae: Molytinae) is a specialist seed predator of Apocynaceae, currently only known to attack *Gomphocarpus sinaicus* (in Sinai) and *Solenostemma argel* (in the Tassili mountains of the southern Sahara)

(Newbold *et al* 2007). There are two generations per year in May and September in Sinai, attacking the two crops of follicles on the host-plant. Adults are mostly active at night, hiding during the day in leaf litter at the base of the plant. The adults make characteristic feeding marks on leaves, cutting the midrib and then feeding distally to the cut; this is a 'latex canal sabotage behaviour' (Zalucki *et al* 2001), a way of reducing the impact of the plant's defensive reaction by preventing the milky alkaloid-laden exudates from reaching the feeding site. Eggs are laid in the wall of the seed follicle, and the larvae penetrate fully to feed on the developing seeds, completely destroying all seeds within the follicle. Pupation occurs in the drying follicle and the emerging adult cuts a large exit hole.

Predators include a number of ladybird species (Coleoptera: Coccinellidae) including *Adonia variegata* (Goeze), present as both larvae and adults, and a number of hoverfly species (Diptera: Syrphidae) including *Eupeodes* (*Metasyrphus*) *corollae* (Fabr.) and *Scaeva pyrastri* (L.), present on *Gomphocarpus* as larvae. Numbers of predators except ladybirds were too low to be useful statistically. Other species likely to be present but not studied here include various bird predators and parasitoids of one or more of the insect species.

We examined use of *Gomphocarpus sinaicus* by herbivores by means of sets of transect surveys during June, July and August 2005. All involved a 2.5-km transect along the bottom of Wadi Arbaein. The transect ran along the path at the wadi bottom and all milkweed plants within 5 m either side of the path were included. In total, 100 plants were sampled (initially 50, and then 50 more were added in July and August), and these were sufficiently far apart for us to treat them as independent replicates because no effects of distance were detectable: thus distance effects are not considered further here. We repeated the transect five times, three times in the morning between 0745 and 1030 h (local time), and two times in the afternoon between 1800 and 1930 h. All replicates were carried out within four days in each month.

On each plant a set of morphological variables were recorded: the height of the plant (as maximum height of stems), the longest horizontal dimension (plant "length"), and the maximum horizontal length perpendicular to this (plant "width") [these were all multiplied together to give plant 'volume']; the proportion of stems on a plant that were dried and senescent; the number of follicles and the number of "large" follicles (>5 cm long - 30% of 1418 follicles), follicle length and width; follicle stage (0-5 from budding [0] to dehisced and dried [5]); distance to the nearest milkweed plant (which could include plants not being sampled on the transect) up to a maximum of 5 m away, after which the nearest neighbour was classified as >5m (31 of the 50 plants). These measurements were taken at the beginning and end of the study period, allowing us to estimate the growth in volume over the summer. Finally, for a subset of 28 plants, we also collected follicles and leaves for chemical analysis (see below).

For each plant we recorded the abundance of each kind of insect on each sampled plant, with sex being identified from external morphology where possible. For each plant, a sampling effort of 4 mins was used. Absolute numbers were recorded except for the oleander aphid, where a four-point ordinal scale was used (no aphids present, aphids on less than 50% of stems, aphids on 50% or more of stems, or the remnants of previous infestations - feeding damage, remains of honeydew, aphid carcasses), and weevil damage (on a four-point subjective scale). Here we use the most complete set of abundance data from one sampling period (August) to avoid problems of seasonal variation in all measured variables. This is reasonable for all taxa except coccinellids, whose abundance had fallen greatly by then (see Results).

For the analysis of plant chemistry, in June we collected approximately five leaves and five follicles from 28 plants and freeze-dried them at -80˚C within 24 hr of collection. Prior to analysis, the plant material was thawed and then weighed. For each plant we combined individual leaves, and individual follicles, such that each plant yielded one data point per leaf or follicle. Plant compounds were extracted by soaking the leaves or follicles in methanol overnight, before being ground-up, filtered and mixed with diatomaceous earth (Celite, MSDS C1628) powder. We then placed the plant material on the top of silica gel for vacuum liquid chromatography (Pelletier *et al* 1986). To isolate and identify the various constituent compounds, we passed different solvents with increasing polarity (hexane, ether, ethyl acetate, chloroform and methanol) through each sample to isolate polar compounds, non-polar compounds, and cardenolides. The eluants were subjected to reverse-phase semi-preparative high-pressure liquid chromatography (HPLC) eluted with methanol: acetonitrile: water (3: 2: 5) at a flow-rate of 0.8 ml min^{-1}, with UV detection at 220 nm and 0.5 sensitivity, using column Ace-C18. This was repeated for two sub-samples from each plant for both leaves and follicles to identify glycosides qualitatively and quantitatively. The cardenolides were identified as a single compound (7,8- rather than 5,6-dehydrocalotropine), and therefore for quantitative estimation the pure compound was subjected to HPLC at various concentrations, and each plant sample fraction compared and standardized to the pure compound. These different plant eluants were thereafter dried and weighed, and concentrations of each calculated in mg per mg of plant material.

Given the multiple intercorrelated variables recorded from the same plants, we analysed the plant-related variables with Principal Components Analysis (PCA) to reduce them to their main independent features - the Principal Component (PC) axes. We used SPSS version 15 to do the analysis, and used the correlation rather than the covariance matrix. Because full chemical data was only known for 27 plants (one plant had no follicles), we replaced missing values by the mean so as to be able to use the full set of morphological data (n=100 plants). This is the reason why plots of correlations in the Results do not show the full range of negative and positive values of a particular PC axis. We also used PCA to pick out the main features of the faunal assemblage. Since *Spilostethus* nymphs hatch and remain together as a batch of up to 60, we recorded their abundance in batches rather than as individuals.

Table 1: Summary statistics of the variables measured during this study. '%' = % zeroes

Variables measured			**N**	**Min**	**Max**	**Mean**	**SE**	**%**	**CV**
Plants	height (cm)		100	23	141	62.3	1.63		26
	volume (dm³)		100	28.4	3787.5	438.4	51.71		118
	% new stems		100	10	100	70.8	2.47		35
	number of follicles		100	0	165	26.5	2.39	9	90
	number of large follicles		100	0	100	9.1	1.69	22	185
	follicle length (cm)		100	2	7.2	4.8	0.09		18
	follicle width (cm)		100	0.75	5	1.7	0.06		37
	mean follicle stage		100	0	5.25	4.1	0.07		18
leaf	cardenolide		27	7.4	179.8	59.2	6.85		60
	polar glycosides		27	14.6	75.3	56.4	2.59		24
	non-polar glycosides		27	228.4	674.1	376.3	21.59		30
follicle	cardenolide		27	19.2	390.8	133.8	24.37		95
	polar glycosides		27	10.1	69.1	46.6	3.17		35
	non-polar glycosides		27	246.8	605.3	400.5	18.63		24
ecology	Nearest-neighbour distance (cm)		50	47	500	405.5	20.69		36
Fauna	*Spilostethus* adults	June	50	0	17	1.6	0.50	72	225
		August	100	0	8	0.4	0.13	88	330
	Spilostethus nymphs	August	100	0	64	2.2	0.81	72	366
	Aphis nerii (score)	June (3-pt scale)	50	0	2	0.9	0.10	34	85
		August (4-pt scale)	100	0	3	1.2	0.30	21	252
	Paramecops	August	100	0	15	2.2	0.30	43	137
	Coccinellidae	June	50	0	103	10.6	2.76	34	185
		August	100	0	3	0.1	0.04	97	619

Results

The measured variables showed great variability among plants (Table 1), especially in size and reproductive output (follicles), as is normal in a population with healthy regeneration. High coefficients of variation were also recorded for cardenolide concentrations (Table 1). Not surprisingly, the abundances of the various taxa of insects were usually more variable than plant morphological or chemical features, and each taxon was not present on many of the plants (Table 1). There were significantly greater concentrations of glycosides in the follicles than in the leaves (paired t test: $t_{25} = 3.0$; $p < 0.01$; see Table 1), and there was no correlation between the concentrations in the leaves and in the follicles within plants ($r = -0.04$; d.f. = 27; p = 0.83).

Multivariate analysis of the plant-related variables (Table 2) shows six axes whose eigenvalues exceed a value of 1.0 (Table 2a), with the first three containing more than half of the variation in the data. Inspection of the correlations with the original variables (Table 2b) shows that the first axis (24% of the variation) represents overall plant size, which also has low correlations with leaf (positively with polar glycosides, negatively with cardenolide and non-polar glycosides) but not follicle defence chemical content. The second axis (16%) concerns independent variation in the balance of defensive chemicals, the concentration of leaf cardenolide and a tradeoff between polar and non-polar glycosides in both leaves and follicles. The third axis (12%) again represents independent variation in defence chemistry, a tradeoff between follicle cardenolide and follicle polar glycosides. Other axes represent only small amounts of variation, and are ignored here.

Analysis of the faunal assemblages on each plant (Table 3) shows two axes with eigenvalues greater than unity (Table 3a), together containing more than 50% of the variation in the data. The first axis accounts for 36% of the variation in the data, and represents (Table 3b) the covarying abundances on plants of weevils (with levels of weevil damage) and milkweed bug nymphs, and to a lesser extent oleander aphids. The second (with 18% of the variation) represents the negative relationship between the abundances of adult milkweed bugs and coccinellids.

We then looked for relationships between the axes of variation in each dataset (Table 4). Both faunal axes were correlated positively with the first plant PC axis (plant size and leaf defences). The sign of the correlations means that there were more of all insect taxa on larger plants, whose leaves also tended to have more polar glycosides but fewer of the other chemical defences (Fig 1). Neither of the faunal axes was correlated significantly with the second plant PC axis. There was a correlation between the first faunal PC axis (milkweed weevils, bug nymphs and oleander aphids) and the third plant PC axis, which represents the chemical defences of the follicle: thus there were more milkweed weevils, batches of milkweed bug nymphs and oleander aphids on plants with high levels of polar glycosides and low levels of cardenolides in their follicles (Fig 2).

Table 2: Principal Components Analysis of the plant-related variables
(a) Eigenvalues

Axis	Eigenvalues	% variance	cumulative%
1	4.408	24.5	24.5
2	2.964	16.5	41.0
3	2.075	11.5	52.5
4	1.389	7.7	60.2
5	1.238	6.9	67.1
6	1.157	6.4	73.5

(b) Correlations between scores along the Principal Axes and the original variables. The defence chemicals are all measured as concentrations (mg per mg plant material).

Original variables	**PCA1**	**PCA2**	**PCA3**	**PCA4**	**PCA5**	**PCA6**
Initial plant size (cm³)	0.836	0.028	0.001	-0.255	-0.004	0.215
Initial number of follicles	0.821	0.264	0.034	0.061	-0.189	-0.254
Initial number of large follicles	0.730	0.181	-0.066	0.112	0.039	-0.467
nearest-neighbour distance (cm)	0.316	0.218	0.077	-0.181	0.200	0.145
Final plant size (cm³)	0.766	0.027	-0.090	-0.369	-0.035	0.208
Proportion of new stems	-0.015	0.061	-0.012	0.716	-0.310	-0.183
Final number of follicles	0.795	0.161	0.244	0.042	-0.185	0.000
Final number of large follicles	0.793	0.231	0.168	0.129	-0.145	0.225
Mean follicle length (mm)	0.310	0.464	-0.092	0.361	0.485	0.179
Mean follicle width (mm)	0.065	0.261	-0.123	0.485	0.541	0.380
Mean follicle stage	0.153	-0.123	-0.304	-0.331	0.448	-0.214
growth in plant size (cm³)	-0.043	-0.197	0.379	-0.190	0.412	-0.255
Leaf cardenolide	-0.296	0.516	0.456	-0.103	-0.100	0.233
Leaf polar glycosides	0.388	-0.785	-0.407	0.164	0.062	-0.034
Leaf non-polar glycosides	-0.370	0.776	0.342	-0.163	-0.043	-0.033
Follicle cardenolide	-0.154	0.225	-0.773	-0.129	-0.249	0.304
Follicle polar glycosides	0.160	-0.592	0.745	0.119	0.105	0.055
Follicle non-polar glycosides	-0.069	0.712	-0.256	-0.034	0.147	-0.491

Table 3: Principal Components Analysis of the faunal assemblage
(a) Eigenvalues

Axis	**Eigenvalue**	**%variance**	**cumulative%**
1	2.172	36.2	36.2
2	1.100	18.3	54.5

(b) Correlations between scores along the Principal Axes and the original variables

Original variables	**PCA1**	**PCA2**
Number of adult *Spilostethus*	0.3012	0.7136
Presence of nymph *Spilostethus*	0.6366	0.0947
Aphid score	0.5343	-0.1492
Number of weevils	0.8085	-0.1102
Weevil damage score	0.7183	0.2958
Number of coccinellids	0.4703	-0.6780

An additional similar analysis used faunal data collected from the initial 50 plants in June, when weevils were not counted but coccinellids were abundant (cf. Table 1). The main features of the data suggested that some plants had more of all insect taxa than others (PC axis 1, 54% of the variation), whilst the second PC axis (36%) suggested that plants with lots of aphids had few milkweed bugs, and *vice versa*. As before, the first axis was correlated with the first plant PC axis ($r_s = 0.41$, n=27, $p<0.05$), whilst the second was close to being significant with the second plant PC axis ($r_s = 0.35$, n=27, p=0.08).

Table 4: Spearman-rank correlations between the plant PC axes (columns) and the faunal PC axes (rows). * = p<0.05 (n=27)

	Plant_1	Plant_2	Plant_3	Plant_4	Plant_5	Plant_6
Fauna_1	0.462 *	0.228	0.478 *	0.064	-0.266	0.113
Fauna_2	0.446 *	-0.244	-0.161	-0.021	0.005	-0.151

Table 5: Axes of variation in the study system

	Faunal abundance		**Plant morphology**		**Plant defensive glycosides**	
	increasing	***decreasing***	***increasing***	***decreasing***	***increasing***	***decreasing***
1	all insects		size number of follicles		leaf polar	leaf non-polar leaf cardenolide
2	(aphids)	(adult bugs)	follicle size		leaf cardenolide non-polar	polar
3	adult bugs	(coccinellids)			follicle polar	follicle cardenolide

Figure 1: Relationships between the abundance of the different taxa of milkweed insects and scores along the first Principal Component of plant-related features: more positive values along this axis denote large plants that tend to have higher concentrations of leaf polar glycosides but lower concentrations of cardenolides and non-polar glycosides.

(a) First Principal Component axis of the faunal abundances (more positive values denote more milkweed weevils, nymphs of milkweed bugs and oleander aphids);

(b) Second Principal Component axis of the faunal abundances (more positive values denote more adult milkweed bugs and fewer coccinellids);

(c) Milkweed weevils *Paramecops sinaitus*; (d) Oleander aphids *Aphis nerii*;

(e) Adult milkweed bugs *Spilostethus pandurus*; (f) batches of nymphs of the milkweed bug *Spilostethus pandurus*.

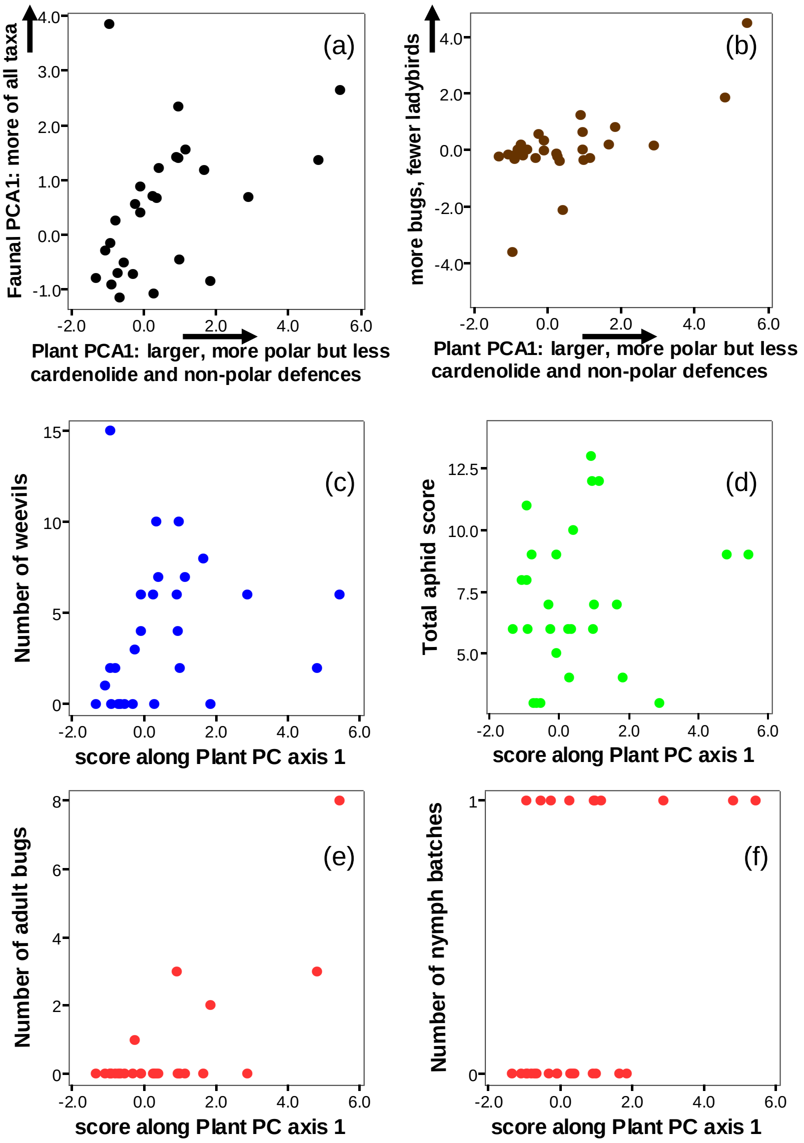
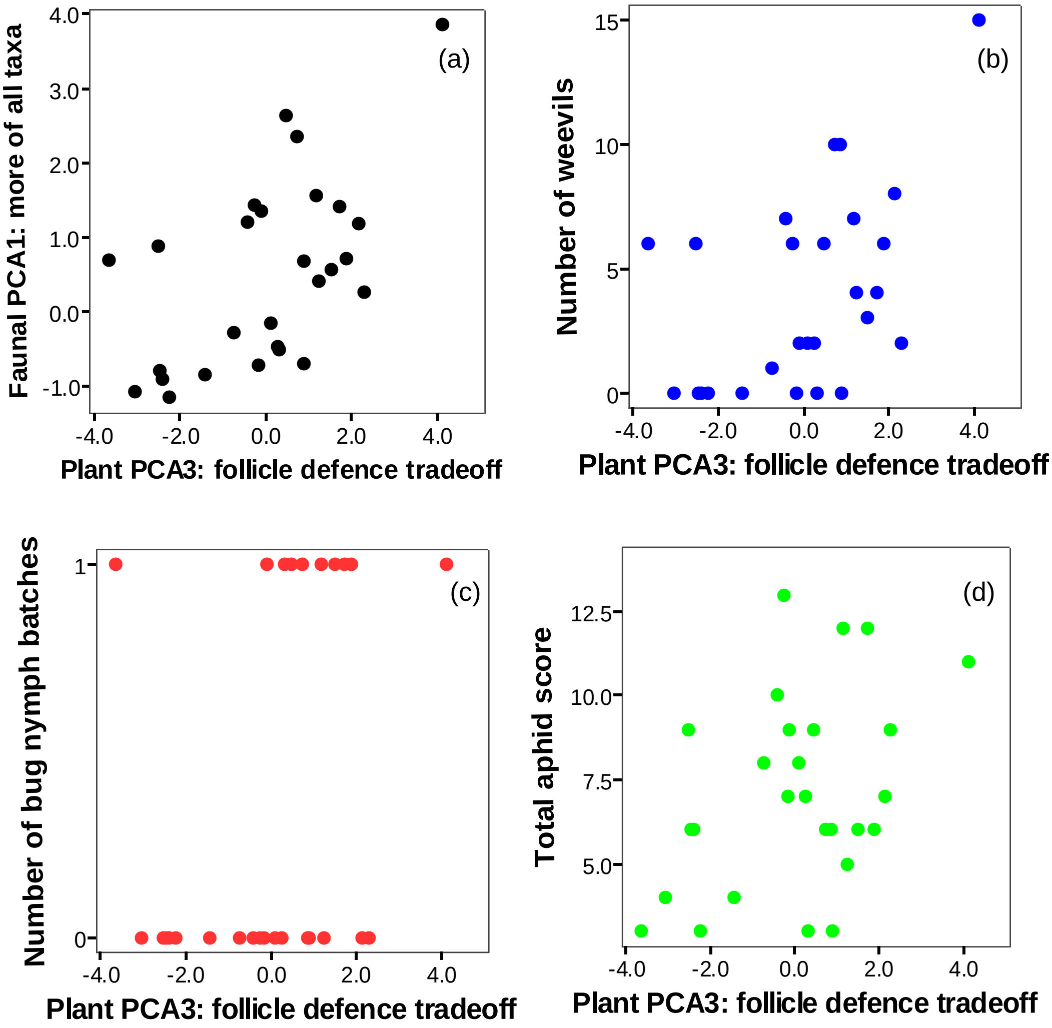


Figure 2: Relationships between the abundance of the different taxa of milkweed insects and scores along the third Principal Component of plant-related features: more positive values along this axis denote plants with higher concentrations of polar gycosides but lower concentrations of cardenolides in their follicles.

(a) First Principal Component axis of the faunal abundances (more positive values denote more milkweed weevils, nymphs of milkweed bugs and oleander aphids);

(b) Milkweed weevils *Paramecops sinaitus*; (c) batches of nymphs of the milkweed bug *Spilostethus pandurus*; (d) Oleander aphids *Aphis nerii*.



Discussion

The North American community of insects on *Asclepias* species consists of 16 species (Table 6). There are remarkable similarities in the life-histories of their Egyptian counterparts, but much needs to be done before we have as clear an idea of their relationships as we have with the North American *Asclepias syriaca*.

In *Asclepias syriaca*, heritable variation in plant properties affects the abundances of many of the various associated insects (Agrawal 2004a, 2005): for example, plant genotypes upon which monarch caterpillars survive well tend to be plants with few attendant ants and few aphids (Mooney & Agrawal 2008). Since plant defence traits form part of this suite of heritable characters, the implication is that plant defensive chemistry impacts on insect herbivores, and attack by herbivores that is selective with respect to plant defence exerts significant natural selection.

Table 6: Comparison between the insect communities specialized to *Asclepias* spp in North America and *Gomphocarpus sinaicus* in Sinai (Betz *et al* 1997, Smith *et al* 2008, Newbold *et al* 2007, this study)

Family	**North American *Asclepias* spp**	**Feeding & life-history**	**Gen**	***Gomphocarpus sinaicus***	**Feeding & life-history**	**Gen**
Aphididae	Oleander aphid *Aphis nerii*	apical leaves, rarely tended, aposematic	?	Oleander aphid *Aphis nerii*	apical shoots & follicles, aposematic	?
	Milkweed aphid *Myzocallis asclepiadis*	dispersed under leaves, untended, not aposematic				
	Asclepiad aphid *Aphis asclepiadis*	apical leaves, ant-tended, not aposematic				
Cuculionidae	Milkweed Stem weevil *Rhyssomatus lineaticollis* (Molytinae)	stem pith (larva); stem juices (adult); adult nocturnal, hiding in leaf litter during day, not aposematic	1	Sinai Milkweed weevil *Paramecops sinaitus* (Molytinae)	seeds (larva); leaves (adult); adult nocturnal, hiding in leaf litter during day, not aposematic	2
	Milkweed weevil *Rhyssomatus annectans* (only *Asclepias incarnata*)	follicles (1st gen larva), stems (2nd gen larva); stem juices (adult)	2			
Coleoptera	Milkweed Leaf beetle *Labidomera clivicollis* (Chrysomelidae)	leaves (adult)	3?	*Adonia variegata,* other polyphagous coccinellids	predator on aphids, but not specific to milkweed	
	Milkweed Longhorn beetles *Tetraopes* spp. (Cerambycidae)	stems/roots (larva); leaves & flowers (adult)	1			
	Milkweed Ladybird *Brachyacantha ursina* (Coccinellidae)	predator on milkweed aphids				
Hemiptera	Milkweed bug *Oncopeltus fasciatus* (Lygaeidae)	developing seeds, aposematic	1	Eastern Seed bug *Spilostethus pandurus* (Lygaeidae)	stem juices & seeds, aposematic	2
	Small Milkweed bug *Lygaeus kalmii* (Lygaeidae)	stem & leaf juices, aposematic	3			
Lepidoptera	Monarch butterfly *Danaus plexippus* (Nymphalidae, Danainae)	leaves, flowers (larva); larva aposematic	1	Plain Tiger *Danaus chrysippus* (Nymphalidae, Danainae)	leaves (larva)	1
	Milkweed Tiger moth *Euchaetias egle* (Arctiidae)	leaves (larva); larva gregarious, aposematic	2			
	Milkweed moths *Cycnia* spp (Arctiidae)	leaves (larva); larva aposematic	1			
Diptera	Milkweed Leafminer Fly *Liriomyza asclepiadis* (Anthomyzidae)	bloch mines on leaf (larva)	1	*Eupeodes corollae*, *Scaeva pyrastri* (Syrphidae)	larvae are predators of aphids, but not specific to milkweed	
Thysanoptera	*Frankliniella tritici*	flowers	?	none recorded		

Here we lay some of the groundwork for exploring this in a closely related system from the Old World. The genus *Gomphocarpus* is the sister-group to *Asclepias*, and contains about 20 species distributed mostly in Africa, but *G.sinaicus* extend further northwards to Israel, Palestine and Jordan. We have shown that plants vary greatly in their overall levels of cardenolides, and within an individual plant there is more in follicles than in leaves. We interpret this variation in terms of plant defensive strategies by individual plants. Consistent with other studies (Hartley & Jones 1999, Strauss & Zangerl 2002), we expect a trade-off between plant reproductive success and allocation to chemical defence using cardenolides or latex: lower levels of defence should entail greater survival and/or reproduction as long as herbivore attack is low, but lower success in habitats with high densities of herbivores. We expect the knock-on community effects to be significant.

There appears to be significant variation among individual plants in the components of their chemical defences, but the technique used here is rather crude, separating polar and non-polar components. Exploring the variation at the level of individual compounds should reveal much more of the variation among individuals, and its impacts on herbivores.

There were indications of different relationships of the insect herbivores to levels of chemical defences, especially aphids. While weevil and bug densities covaried, those of aphids varied more independently, and possibly inversely. We did not measure the attendance of ants on aphid colonies, a significant omission in the light of the recent study of *Asclepias syriaca* (Mooney & Agrawal 2008), where the impact of ants was a major component.

The suggestive pattern of association between adult *Spilostethus* and *Aphis nerii*, if real, could arise in a number of ways. Most obviously there could be direct competition for feeding sites (Denno *et al* 1995). *Spilostethus* typically feed at the base of the follicle, and heavy aphid infestations cover the whole follicle and the basal stem of the follicle. However, the bugs are mainly feeding from the seeds within the follicles, whereas the aphids are plugged into the phloem: thus competition seems rather unlikely unless mediated indirectly. Alternatively, the two species may have different plant preferences, perhaps most likely associated with the levels of cardenolide glycosides. Even though both sequester these compounds, the optimal level of the toxins may vary for the two species.

There are a number of indirect interactions that might also underlie the observed patterns (van Veen *et al* 2006): for example, bugs and aphids could share one or more predators, such as the coccinellid beetles associated with the aphid colonies. Whilst these predators would not take adult bugs, they would be potential predators of eggs and juveniles. Female bugs in particular may therefore attempt to limit egg and larval predation through their oviposition behaviour (Ballabeni *et al* 2001, Nomikou *et al* 2003, Bond *et al* 2005), preferring to oviposit on plants with few or no aphids. Alternatively, the bugs and aphids may interact indirectly via their effects on the host-plant, altering the chemical profile such that the plants become less optimal for one of the species (Karban & Baldwin 1997, Havill & Raffa 2000, Ode 2006). Given the differences in mobility between an adult bug and an aphid colony, these effects would lead to more rapid behavioural changes in the former, and are consistent with bugs being found on less toxic plants.

There are of course aspects of plant variation other than the cardenolide glycosides that may influence herbivory, such as nutrient quantity or quality (White 1984, Simpson & Raubenheimer 2001, Awmack & Leather 2002, Agrawal 2004b) or structural defence (Karban & Baldwin 1997). In terms of the cardenolides themselves, the differences in levels of cardenolides associated in particular with *Spilostethus* may be the result of differences in the levels of constitutive defence, or they may be due to differences in induced defences, perhaps arising from the feeding activity of different members of the herbivore assemblage (see below; Agrawal 2004b).

Unlike in many other aphid species (Pickett *et al* 1992, Powell *et al* 2006), the abundance of *Aphis nerii* on *Gomphocarpus sinaicus* is not obviously associated with plant chemistry, yet there is a suggestion that aphids are absent from plants with the lowest levels of cardenolides, consistent with their use of plant toxins for defence (Groeters 1993, Martel & Malcolm 2004). Population growth may vary with respect to inter-specific differences in host-plant cardenolide levels, with *Aphis* doing worse on milkweed species with higher toxin levels (Agrawal 2004b), or be unaffected (Martel & Malcolm 2004; see also Groeters 1993, Malcolm, 1990).

Preliminary work suggests that the weevil *Paramecops sinaitus* does not contain *Gomphocarpus*-derived cardenolides, implying that it is able to metabolise these compounds since it certainly cannot avoid them (Elbanna & Shuker, unpublished data). This species is a specialist on *Gomphocarpus*, and can probably detoxify and eliminate the toxins: it is predicted to be unaffected by inter-individual variation in toxin levels among the milkweed plants. It is therefore intriguing that its abundance is positively correlated with plant defensive chemistry, implying an active benefit to the weevil, perhaps in warding off natural enemies or possibly providing extra resources. The issue of whether "specialists" are better able to cope with plant defences is still an open one (Agrawal 2004b; Schoonhoven *et al* 2005), and this study system with its two specialists (*Aphis nerii* and *Paramecops sinaitus*) and one generalist (*Spilostethus pandurus*) offers an opportunity to examine the importance of specialisation on plant-herbivore interactions in a relatively simple ecosystem.

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الملخص العربى

نظام استخدام المصادر الكيميائية فى نبات الحرجل بواسطة الحشرات فى شبه جزيرة سيناء

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تتناول الدراسة علاقة الشكل الظاهرى والمواد الكيميائية التى يفرزها نبات الحرجل فى شبه جزيرة سيناء ومدى علاقة تلك الإفرازات بالعشائر الحشرية نباتية التغذية والتى تتواجد على النبات. أتضح من الدراسة أن الآنواع والعشائر المختلفة من الحشرات تتفاعل بطريقة متباينة ومختلفة طبقا للصفات الرئيسية للنبات ووجد أن المواد الدفاعية التى يفرزها النبات تلعب دورا حيويا فى تحديد تواجد وانتشار أنواع الحشرات ومدى إرتباطها بالنبات. ولقد أوضحت الدراسة أن هناك علاقة وثيقة بين المواد الكيميائية الدفاعية التى يفرزها النبات وبين تواجد أنواع الحشرات من نوع المَن، بينما تباين تواجد كل من أنواع الخنافس والبق النباتى بصورة كبيرة على عكس حشرة المن. أيضا أتضح أن أفراد النبات تتباين بصورة معنوية فى إفرازها للمواد الكيميائية الدفاعية. وآخيرا، تم مقارنة العشائر الحشرية المتواجدة على نبات الحرجل مع المتواجدة على نفس النبات فى شمال أمريكا.