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The impact of leaf shape on the feeding preference of insect herbivores: experimental and field studies with *Capsella* and *Phyllotreta*

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SUMMARY

Leaves display an enormous array of sizes and shapes. Although these attributes appear to have evolved primarily in response to abiotic conditions in the plant's habitat, the importance of insect herbivores as additional selective agents is still poorly understood. A necessary requirement for leaf size and shape to evolve in response to attack by insects is that insects must respond to and/or be affected by, leaf morphology. We tested leaf-shape preferences in adult flea beetles (*Phyllotreta* spp.) feeding on the highly variable rosette leaves of *Capsella bursa-pastoris*. Contrary to theoretical expectation (Brown & Lawton 1991), leaves with deeply lobed margins were more intensely damaged, both in field-collected and experimental plants. In two ancillary experiments with *Capsella*, we found that *Spodoptera* caterpillars showed no preferences for leaf shape, but that adult vine weevils (*Otiorhynchus sulcatus*) did, preferring (as predicted), undivided over divided leaves. We conclude that Brown & Lawton's (1991) hypothesis is at best weakly supported by laboratory data for vine weevils, refuted by laboratory data for *Spodoptera*, and consistently refuted by both laboratory and field data for flea beetles. Although the experiment tried to reduce confounding variables to a minimum, interpretation was complicated by correlations between leaf shape and other developmental parameters of the plants, and highlights the difficulty of disentangling leaf-shape effects from other confounding factors.

1. INTRODUCTION

Leaves in nature display an enormous array of sizes and shapes, commonly believed to have evolved in response to the abiotic conditions of the plant's habitat (Weaver & Clements 1938; Talbert & Holch 1957; Vogel 1970; Parkhurst & Loucks 1972; Givnish 1979; Gates 1980). However, as Brown & Lawton (1991) pointed out, the importance of herbivores as selective agents in the evolution of leaf size and shape has rarely been investigated and is still poorly understood. They suggested several physical characteristics of plant foliage, including size and shape, that may have arisen as adaptations against vertebrate and invertebrate herbivores. The most compelling examples come from studies of plant–vertebrate interactions (Brown & Lawton 1991; Givnish *et al.* 1994); the role of insect herbivores in the evolution of leaf size and shape has been relatively neglected.

A growing body of evidence shows that aspects of leaf morphology influence leaf choice and efficiency of use by insects. These include visual appearance (Prokopy & Owens 1983), waxiness (Stork 1980; Edwards 1982; Woodhead & Chapman 1986; Edwards & Wanjura 1991; Bodnaryk 1992*a*, *b*), toughness (Tanton 1962; Edwards & Wanjura 1991; Matsuki & MacLean 1994) and the presence or absence of trichomes (Schillinger & Gallun 1968; Southwood 1986; van Lenteren & de Ponti 1990; Dakshayani *et al.* 1993; Palaniswamy & Bodnaryk 1994). Comparatively little is known about how leaf shape influences an insect's choice of feeding or oviposition sites, although it is a factor that may affect feeding rate or limit access to certain areas (Brown & Lawton 1991). Although detailed studies are few, a small number conclude that insects have been the selective pressure behind the evolution of particular leaf characteristics, such as mimicry (Gilbert 1982; Niemela & Tuomi 1987), differences in leaf shape between plant species sharing a habitat (Rausher 1978; Gilbert 1982) and differences between juvenile and adult foliage (Gilbert 1982).

Comparisons between plant species of the ways in which leaf shape might influence the choice and performance of insect herbivores may be confounded by inevitable physiological and biochemical differences between the leaves of different plant species. To minimize these problems, we studied adult flea beetles (Coleoptera: Chrysomelidae) in the genus *Phyllotreta* (*P. atra* (Fab.), *P. undulata* Kutschera and *P. nigripes*

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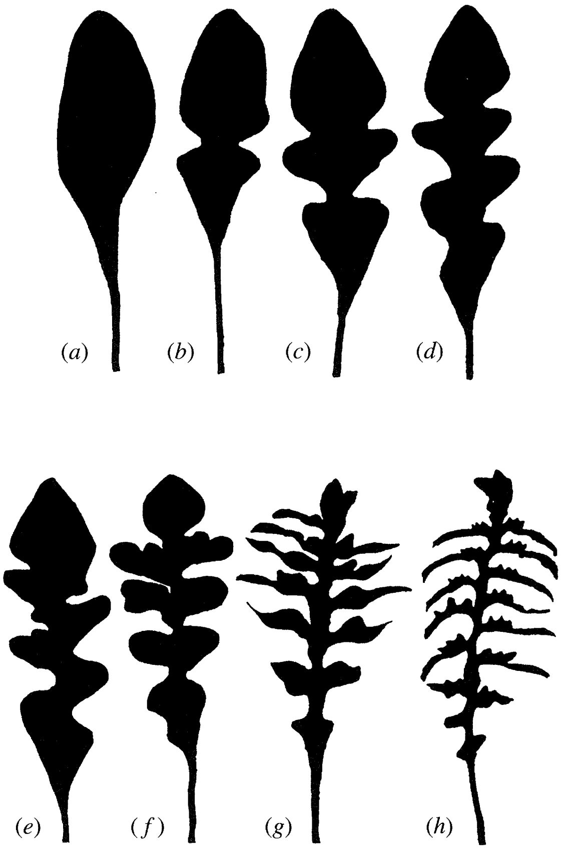

Figure 1. The diversity of rosette leaf shapes in *Capsella bursa-pastoris*. Leaves were divided into five types according to the degree of indentation (lobing) of the margin. (*a*) Type 1: entire, with no indentations. (*b*) Type 2: one shallow indentation on either side, resulting in two round lobes. (*c*, *d*) Type 3: two or three shallow indentations on either side, resulting in three or four round lobes. (*e*, *f*) Type 4: three or more deep indentations (virtually reaching the midrib) on either side, resulting in several deep round lobes. (*g*, *h*) Type 5: deeply pinnatifid, with more than five deep indentations reaching the midrib on either side, resulting in numerous long, narrow lobes.

(Fab.)), feeding on the rosette leaves of a single species of plant, *Capsella bursa-pastoris* (L.) (Brassicaceae). *Capsella* displays enormous intra-specific variability in the shape of its rosette leaves, which may range from entire to deeply pinnatifid within the same individual (figure 1). Adult *Phyllotreta* are specialists on Brassicaceae and can cause heavy damage to *Capsella* in the field, leaving characteristic feeding holes in the leaf surface. The three species cause identical damage, and we have not distinguished between them. Herbivory by flea beetles is known to decrease plant growth (James *et al.* 1992; Bach 1994*a*, *b*) and flower production (James *et al.* 1992), and to increase plant mortality (Bach 1994*b*) in a range of species, although to our knowledge no studies have been conducted on their effect on *Capsella bursa-pastoris*.

We are not suggesting that flea beetles have driven the evolution of variation in leaf shape in *Capsella*. Rather, if leaf shape can be shown to alter herbivore preference and/or performance, then this is consistent with (but does not prove – Nuñez-Farfán & Dirzo 1994) the hypothesis that herbivory has played a role in the evolution of leaf shape (Brown & Lawton 1991). Several studies reviewed by Brown & Lawton point to the conclusion that reduced herbivory may be one selective advantage of highly divided leaves. We carried out a range of observations and experiments to test this hypothesis, concentrating on herbivore preference assessed by feeding damage. The main results are presented in three sections (field sampling, field experiment and laboratory experiments), each with its own methods and results sections. Some preliminary results with two other species of insect herbivores are also presented in §5. Diametrically opposite to our main prediction, we find consistent evidence that highly divided leaves in *Capsella* suffer more, not less, damage from flea beetles. We conclude by briefly discussing the difficulties of experimentally testing the impacts of leaf size and shape on insect herbivores, unconfounded by other variables.

2. STATISTICAL ANALYSIS

Data were analysed using generalized linear models in GLIM 4 (McCullagh & Nelder 1983; Aitkin *et al.* 1989; Crawley 1993). The significance of an explanatory factor in GLIM is assessed by deleting it from, or fitting it into, the model containing all other explanatory variables and interactions, and analysing the resulting change in deviance (Crawley 1993). GLIM allows the specification of normal, Poisson or binomial error distributions. With Poisson and binomial error distributions, the change in deviance attributable to a given factor is distributed approximately as chi-squared. When either of these error structures are used, a heterogeneity factor (*HF*) is calculated (residual deviance over residual degrees of freedom) after the full statistical model has been fitted. Values of *HF* larger than one indicate overdispersion which can lead to spuriously significant results. High heterogeneity factors have been corrected by scaling the deviance by *HF*, or by transforming the response variable that produces more conservative parameter estimates (Crawley 1993). Other aspects of the statistical analyses are dealt with in each section.

3. FIELD SAMPLING

(*a*) *Methods*

Plants were collected from an area of ex-arable land (Silwood Bottom, Berks, U.K.) on 1 June 1995 to determine the level of damage caused by adult flea beetles (predominantly *P. atra* and *P. undulata*, with some *P. nigripes*) in wild populations. We collected all the rosette leaves from 15 randomly sampled plants; allocated them to types 1–4 (figure 1) (there were no type 5 leaves in the collection); measured the maximum length and width of each leaf; and counted the number of characteristic damage holes caused by adult flea beetles. In these field samples only, we also collected all the stem leaves, and treated them in a similar way. Stem leaves resemble type 1 rosette leaves but have a basal auricle that clasps the stem (Clapham *et al.* 1987). In total in these field samples we examined 119 rosette and 155 stem leaves.

Table 1. *Summary of the results from random field sampling of 15 plants using 'damaged/undamaged' and 'damage level' (number of damage holes) as alternative response variables*

(LT, leaf type; LS, leaf size (length × width); Int, interaction; (S), change in deviance resulting from fitting the explanatory variable separately into the model; (D), change in deviance resulting from deleting the explanatory variable from the full model; *F* values when the model was fitted with normal errors and χ^2 values when the model was fitted with binomial errors.)

	Damaged/undamaged			Damage level[a]		
	LT	LS	Int	LT	LS	Int
(S)	$\chi^2 = 82.88^{***}$	$\chi^2 = 108.3^{***}$	—	$F = 52.45^{***}$	$F = 174.52^{***}$	—
(D)	ns	$\chi^2 = 37.07^{***}$	ns	—	—	$F = 6.21^{***}$

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[a] The response variable was square root transformed due to high heterogeneity factors.

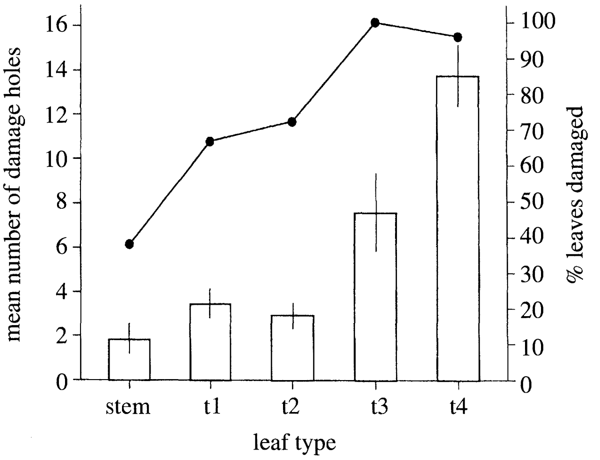

Figure 2. Mean (±95% confidence limits) number of damage holes for stem leaves, and for each of four types of rosette leaves (bars) and the total proportion of leaves damaged (dots and solid lines) for randomly sampled field plants. (t1 = type 1 leaves etc., see figure 1. Stem leaves resemble type 1 leaves).

(*b*) *Results*

We analysed the leaves in two ways, first using 'damaged' or 'undamaged' as a simple binary response variable, and second by quantifying the amount of damage. We used leaf size (length × width) and morphotype (1–4) as independent explanatory variables (table 1). Leaves with medium to deeply lobed margins (types 3 and 4) are larger and, contrary to our main hypothesis, suffer more damage than leaves with entire or slightly lobed margins (stem leaves and leaves of types 1 and 2) (table 1, figure 2). While there is an unambiguous effect of leaf size on the probability of a leaf being damaged, no effect of leaf type independent of leaf size can be detected.

4. FIELD EXPERIMENT

(*a*) *Methods*

The field experiment and the greenhouse experiments that follow (§5), all used seeds from the same seed stock ('Emorsgate', Norfolk, U.K.).

Capsella seeds were sown in the greenhouse between May and June 1995 and the young plants were transplanted into Silwood Bottom approximately six weeks after sowing when they had rosettes with between five and 10 well-developed leaves but no flowering stems. The area had been rotivated a month before the beginning of the experiment and was virtually clear of vegetation. The surrounding wild and cultivated plants maintained a high population of all three species of adult flea beetles throughout the summer.

Table 2. *Damage scores in the field experiment according to the proportion of leaf area damaged by flea beetles and geometric mean of each leaf area damage interval*

score	% leaf damaged	geometric mean %
0	0%	0
1	1–5%	2.6
2	6–15%	10.09
3	16–25%	20.30
4	26–50%	37.30
5	51–75%	62.58
6	> 75%	87.70

We randomly selected ten 50 × 50 cm experimental blocks. Within each block, we planted four rows of four plants, separated by 10 cm from each other and 10 cm from the edge of the block. Plants, which at this stage exhibited a great variety of leaf shapes both within and between individuals, were allocated at random to the blocks. Three leaves were randomly selected on each plant, allocated to morphotype, measured and marked with coloured sewing cotton loosely tied around the petiole, and then regularly monitored. In total, 480 leaves were marked (10 blocks, 16 plants per block, three leaves per plant), constituting the 1st cohort. Thereafter, the 2nd and 3rd cohorts were established at weekly intervals using eight plants selected at random in each of the 10 blocks; we marked and recorded the fate of three randomly chosen leaves per plant (a total of 240 leaves). Two weeks after the 3rd cohort had been marked, some of the plants produced a 4th cohort of leaves; this sample contained only 111 leaves: 1–3 leaves per plant in 1–8 plants per block, depending on the availability of leaves.

For two months (July and August), the size of the leaf (measured as leaf length) and the damage caused by flea beetles were recorded weekly. No individual leaf survived for more than five weeks in the field. Damage was scored non-destructively (using calibrated drawings) as a proportion of leaf area (table 2).

Table 3. *Summary of the results for the field experiment using damaged (dam)/undamaged (undam), 'last', 'average' and 'rate' as alternative response variables (see Section 4 for details). LT: leaf type, LS: leaf size (measured as length), Int: interaction;* (S) *Change in deviance resulting from fitting the explanatory variable separately into the model,* (D) *Change in deviance resulting from deleting the explanatory variable from the full model.* F *values when the model was fitted with normal errors and* χ^2 *values when the model was fitted with Poisson or binomial errors.*

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

leaf		dam/undam			last			average			rate		
cohort		LT	LS	Int	LT	LS	Int	LT	LS	Int	LT	LS	Int
1st	(*S*)	ns	$\chi^2 = 18.89^{***}$	—	$\chi^2 = 10.57^*$	$\chi^2 = 17.76^{***}$	—	F = 6.82***	F = 38.18***	—	ns	F = 25.58***	—
	(*D*)	ns	$\chi^2 = 21.85^{***}$	ns	ns	$\chi^2 = 13.38^{***}$	ns	ns	F = 31.22***	ns	ns	F = 20.81***	ns
2nd	(*S*)	ns	ns	—	ns	ns	—	ns	ns	—	ns	ns	—
	(*D*)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3rd	(*S*)	ns	ns	—	ns	$\chi^2 = 5.89^*$	—	ns	F = 5.49*	—	ns	F = 6.54*	—
	(*D*)	—	—	$\chi^2 = 31.8^*$	ns	$\chi^2 = 3.91^*$	ns	ns	ns	ns	ns	F = 4.96*	ns
4th	(*S*)	ns	ns	—	ns	ns	—	ns	ns	—	ns	ns	—
	(*D*)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Because we selected leaves on each plant at random, and because each *Capsella* plant produces a mixture of rosette leaves of different shapes, we had no control over the number of replicates per treatment (where 'treatment' is leaf shape). Such non-orthogonal data is easily handled in GLIM by assessing the change in deviance of the explanatory variables when they are removed from (and fitted back into) the maximal model (i.e. the model including all the other explanatory variables and their interactions) (Crawley 1993), which in this case included block effects, plant effects, leaf type and leaf size. Type 5 leaves appeared in only a few plants (three in the 1st cohort, six in the 2nd cohort, 12 in the 3rd and 12 in the 4th); we therefore combined type 4 and 5 leaves in the analysis.

Analyses were carried out separately for each of the four leaf cohorts using several response variables: damaged/undamaged; the last (i.e. maximum) damage score per leaf; and the average (i.e. mean) damage score acquired by the leaf throughout its life. Using the geometric mean damage score (table 2) we also calculated a fourth response variable, the rate of damage, by dividing the maximum proportion of leaf damaged by the number of weeks that the leaf survived.

(*b*) *Results*

In the 1st cohort 'last' and 'average' damage scores of type 3 and 4 leaves are significantly higher than those of type 1 and 2 leaves, when leaf type is fitted separately into the model (table 3). However, leaf type is not significant when deleted from the model containing leaf size, because as in §3, there is a correlation between leaf type and size (leaves with deeply lobed margins are significantly longer than leaves with entire margins – 1st: $F = 25.76$, $p < 0.001$; 2nd: $F = 4.09$, $p < 0.01$, 3rd: $F = 10.61$, $p < 0.001$). The 4th cohort did not survive long enough to analyse effectively). Leaf size repeatedly appears as the only explanatory variable causing a significant change in deviance when deleted from the full model (in addition to the expected block and plant effects, which for simplicity have not been included in table 3). In other words, the probability of a leaf being damaged, the proportion of the leaf area damaged and the rate of increase in damage, all increase with leaf length and hence indirectly with increasing leaf division.

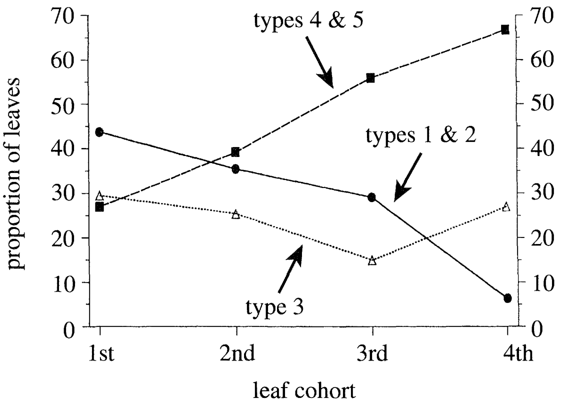

Figure 3. Changes in the proportions (as a percentage) of different types of rosette leaves in leaf cohorts 1–4 of the field experiment.

The higher proportion of area damaged in type 4 leaves, compared with type 1 leaves, is the result of a real increase in damage and is not an artefact of differences in leaf area. Type 4 leaves are consistently longer than type 1 leaves and their estimated area is either larger than or equal to that of type 1 leaves (1st: $t = 4.68$; $p < 0.001$, 2nd: $t = 0.32$, $p > 0.05$; 3rd: $t = 1.30$, $p > 0.05$).

However, this experiment highlights some complicating morphological and phenological factors that may influence the results. (i) Leaves with deeply subdivided margins are much commoner later in the season than leaves with entire or superficially lobed margins (figure 3). (ii) In the first two cohorts, leaves with deeply lobed margins survived longer than leaves with entire or superficially lobed margins (1st: $F = 89.21$, $p < 0.001$, 2nd: $F = 5.34$, $p < 0.01$). (iii) There are differences in the relative position of the leaf morphotypes within the rosette; leaves with deeply lobed margins tend to lie on the top of the rosette, thus partially covering the leaves with entire or superficially lobed margins. We return to these potentially confounding variables in the discussion.

5. LABORATORY EXPERIMENTS

(*a*) *Methods*

We used freshly excised *Capsella* leaves from greenhouse-grown plants to test herbivore preferences. In addition to trials with adult flea beetles, we tested adult vine weevils (*Otiorhynchus sulcatus* Fab., Coleoptera: Curculionidae) and larvae of *Spodoptera exigua* Hub. (Lepidoptera: Noctuidae). Vine weevils and 9-day-old *Spodoptera* larvae feed mainly from the edge of the leaf inwards, although some central damage holes were observed; younger, 2–5-day-old *Spodoptera* larvae make small holes in the lamina. All experiments were performed in a controlled temperature (CT) room (20±2.5 °C, 70±5% relative humidity, 16:8 h light:dark photoperiod).

(*i*) *Flea beetles*

We used transparent, ventilated plastic boxes (13 × 6 × 7.5 cm) to present type 1 and type 4 leaves in pairs to *Phyllotreta*; leaves were inserted at an angle into small squares of moist 'Oasis' (a water-absorbent material used for cut flowers) glued to the base of each box, separated by approximately 6 cm. We standardized leaves for size (mean length±s.e. (standard error): 8.16±0.34 cm). Five freshly collected adult *Phyllotreta undulata* were released into each box and the feeding damage was assessed after five days ($n = 50$).

(*ii*) *Vine weevils*

Adult vine weevils came from cultures kept at Silwood Park. Pairs of leaves (one type 1 and one type 4; mean length±s.e.: 7.5±0.14 cm) were presented side by side to single weevils in petri dishes (9 cm diameter). Leaf area eaten was assessed after 20 h ($n = 28$). In a second experiment ($n = 20$) (mean leaf length±s.e.: 7.15±0.13 cm), the damage was assessed every 8 h for a maximum of 48 h.

(*iii*) Spodoptera

Spodoptera larvae were obtained from Dr Tanya Wright at Zeneca. One (9-day-old) or three (2–5-day-old) larvae were tested in a petri dish with a pair of leaves (one type 1 and one type 4; mean leaf length±s.e.: 7.6±0.16 cm), and the damage was recorded after 20 h ($n = 20$ for each age group).

(*b*) *Results*

(*i*) *Flea beetles*

There was no significant difference in damage levels between type 1 and type 4 leaves ($t = 1.94$, $p > 0.05$), although overall damage levels were very low (mean number of damage holes±s.e. were 0.78±0.15 (type 1 leaves) and 0.44±0.13 (type 4 leaves)).

(*ii*) *Vine weevils*

The damage caused by vine weevils was large, with as much as 15% (20 h experiment) to 45% (48 h experiment) of the leaf area being removed by the end of the trial. In the 20 h experiment, type 1 leaves were significantly more damaged than type 4 leaves ($t = 3.08$, $p < 0.01$). Similarly, in the 48 h experiment, the cumulative damage to the leaves during the first 16 h was significantly larger for type 1 leaves than for type 4 leaves ($t = 2.12$, $p < 0.02$), although this difference disappeared in further recordings (24 h: $t = 1.81$; 32 h: $t = 1.87$; 40 h: $t = 1.74$; 48 h: $t = 1.60$; all $p > 0.05$). These results are the first to show more damage in entire (type 1) leaves compared with deeply divided (type 4) leaves.

(*iii*) Spodoptera

Nine-day-old *Spodoptera* consumed up to 90% of the leaf area. Damage by 2–5-day-old larvae never exceeded 15% of the leaf area. There were no significant differences between type 1 and type 4 leaves in the total leaf area consumed (9-day-old, $t = 0.36$, $p > 0.05$; 2–5-day-old, $t = 0.006$, $p > 0.05$).

6. DISCUSSION

Brown & Lawton (1991) postulated that the evolution of highly divided leaves may be one of the strategies employed by some species of plants to reduce attack by insect herbivores. They summarized a smattering of evidence consistent with the hypothesis that highly divided leaves can reduce the foraging efficiency of insect herbivores, or make effective feeding impossible. Under these circumstances, insects would be expected to: (i) show a preference for entire leaves and/or (ii) perform better on entire leaves than on deeply lobed or highly subdivided leaves. The present study is the first, direct experimental test of Brown and Lawton's hypothesis, concentrating on herbivore preference.

We chose to work primarily with adult flea beetles in the genus *Phyllotreta*, and a single species of plant, *Capsella bursa-pastoris*, for two reasons. First, individual *Capsella* plants support an exceptional array of leaf shapes (figure 1), greatly reducing the confounding variables that must arise in assessing herbivore preference for different species of plants that differ in leaf shape. Second, there is some evidence that the behaviour of adult flea beetles is affected by the architecture of host leaves. In *Phyllotreta cruciferae*, convolutions in the leaves of kale (a cultivar of *Brassica oleraceae*) appear to interfere with walking between feeding sites. As a result, foraging flea beetles are more likely to leave kale plants than they are to leave collards (a *B. oleraceae* cultivar with flat leaves) (Vaughn & Hoy 1993).

We asked whether adult flea beetles (*Phyllotreta* spp.) preferred entire rosette leaves of *Capsella bursa-pastoris* compared with highly subdivided leaves of the same species, assessing preference by the amount of feeding damage. Contrary to our hypothesis, in laboratory feeding trials the beetles showed no preference, whilst in plants collected from the field and from an experimental garden, leaves with deeply lobed margins were *more* intensely damaged. However, because leaf shape and leaf size were correlated, the significance of leaf shape *per se* disappeared when leaf length was included in the statistical models. In two ancillary experiments with *Capsella*, we found that *Spodoptera*

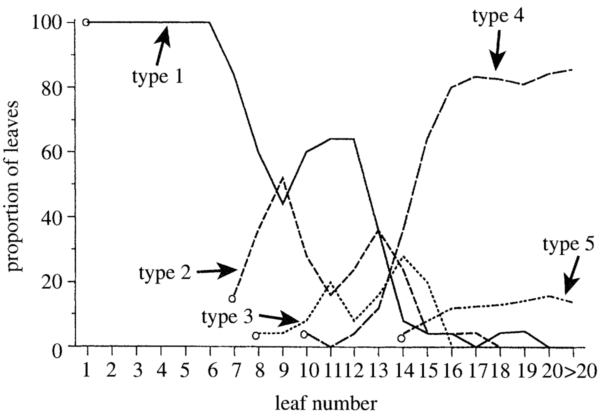

Figure 4. Changes in the relative proportions (as a percentage) of type 1–type 5 rosette leaves for 25 *Capsella* plants grown in a greenhouse for two months, from germination until flowering. 1 indicates the first leaf to appear through to the 20th leaf and beyond. Plants were examined every day. Seedlings were kept in the original seed trays until they reached the five-leaf stage, when their root systems had developed enough to be transferred individually to pots (11 cm diameter, 9.5 cm depth). The greenhouse had natural light; temperatures ranged from approx. 20 °C (daytime) to 10 °C (night-time).

caterpillars showed no preferences for leaf shape, but that adult vine weevils did, feeding more heavily (as predicted) on undivided leaves.

On the basis of these results, we have to conclude that Brown & Lawton's (1991) hypothesis is at best weakly supported by laboratory data for vine weevils, refuted by laboratory data for *Spodoptera*, and strongly and consistently refuted by both laboratory and field data for flea beetles.

There is, however, a complication. Although these experiments reduced confounding variables to a minimum (by focusing on differences in leaf shape within a single host plant species), interpretation of the data is complicated by unexpected correlations found between leaf shape and several other developmental characteristics of the plant (see §4). We have been unable to trace any publications dealing with leaf development patterns in *Capsella bursa-pastoris*, but in the course of the present experiments we discovered several potentially confounding variables.

1. More deeply lobed leaves tend to appear later in the plant's life (e.g. figures 3 and 4). On average, therefore, divided leaves are younger than entire leaves, which may affect herbivore preference and performance via age-related changes in leaf primary and secondary metabolism (McKey 1979; Raupp & Denno 1983; Whitham 1983; Meyer & Montgomery 1987); flea beetles are unlikely to be immune to these influences (Feeny *et al.* 1970; Mitchell 1988; Porter *et al.* 1991; Peng *et al.* 1992; Bach 1993; Vaughn & Hoy 1993; Bartlet *et al.* 1994).

2. Leaves with deeply lobed margins grow at a significantly faster rate and reach a significantly larger size than leaves with entire or superficially lobed margins, confounding leaf shape and size effects (table 3). It is unclear why leaf size affects feeding rates in flea beetles. Increased visual apparency seems unlikely, because host selection in flea beetles is based solely on chemical detection (e.g. Feeny *et al.* 1970; Vincent & Stewart 1984; Peng *et al.* 1992). More likely, faster growing leaves are more attractive, because they have higher concentrations of essential nutrients (Woodwell *et al.* 1975).

3. Leaves with deeply lobed margins differ from leaves with entire or superficially lobed margins in two further ways: position on the plant and life-span. The rosette leaves of *C. bursa-pastoris* grow in a spiral so that the last to appear are highly divided and tend to lie on top of older, less divided leaves, possibly making more divided leaves more accessible to flea beetles. Second, leaves with deeply lobed margins have a longer life-span than leaves with entire or superficially lobed margins (although in the present experiments, longevity *per se* had no detectable effects on flea beetle damage).

Given these potentially confounding variables, it is clear that we have not performed a clean test of the leaf-shape hypothesis. Whether it will be possible to do so with other plant species is a moot point. Confounding variables are likely to be much less within, rather than between, plant species. But they can probably never be entirely eliminated. Understanding the role played by insect herbivores in the evolution of leaf shape would benefit from experimental field studies that examine not only leaf shape but also leaf chemistry, and should involve inter- and intra-specific comparisons and a variety of different herbivores.

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REFERENCES

Aitkin, M., Anderson, B., Francis, B. & Hinde, J. 1989 *Statistical modelling in GLIM*. Oxford: Clarendon Press.

Bach, C. E. 1993 Effects of microclimate and plant characteristics on the distribution of a willow flea beetle, *Altica subplicata*. *Am. Midl. Nat.* **130**, 193–208.

Bach, C. E. 1994*a* Effects of herbivory and genotype on growth and survivorship of sand dune willow (*Salix cordata*). *Ecol. Ent.* **19**, 303–309.

Bach, C. E. 1994*b* Effects of a specialist herbivore (*Altica subplicata*) on *Salix cordata* and sand dune succession. *Ecol. Monogr.* **64**, 423–445.

Bartlet, E., Parsons, D., Williams, I. H. & Clark, S. J. 1994 The influence of glucosinolates and sugars on feeding by the cabbage stem flea beetle, *Psilliodes chrysocephala*. *Ent. exp. Appl.* **73**, 77–83.

Bodnaryk, R. P. 1992*a* Distinctive leaf feeding patterns on oilseed rapes and related Brassicaceae by flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Can. J. Pl. Sci.* **72**, 575–581.

Bodnaryk, R. P. 1992*b* Leaf epicuticular wax, an antixenotic factor in Brassicaceae that affects the rate and pattern of feeding of flea beetles, *Phyllotreta cruciferae* (Goeze). *Can. J. Pl. Sci.* **72**, 1295–1303.

Brown, V. K. & Lawton, J. H. 1991 Herbivory and the evolution of leaf size and shape. *Phil. Trans. R. Soc. Lond.* B. **333**, 265–272.

Clapham, A. R., Tutin, T. G. & Moore, D. M. 1987 *Flora of the British Isles*, 3rd edn, Cambridge University Press.

Crawley, M. J. 1993 *GLIM for Ecologists*. Oxford: Blackwell Scientific Publications.

Dakshayani, K., Bentur, J. S. & Kalode, M. B. 1993 Nature of resistance in rice varieties against leaf folder *Cnaphalocrocis medinalis* (Guenee). *Insect Sci. Appl.* **14**, 107–114.

Edwards, P. B. 1982 Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? *Aust. J. Ecol.* **7**, 347–352.

Edwards, P. B. & Wanjura, W. J. 1991 Physical attributes of Eucalypt leaves and the host range of crysomelid beetles. *Proc. 7th Int. Symp. Insect–Plant Relationships. Symp. Biol. Hung.* **39**, 227–236.

Feeny, P., Paauwe, K. L. & Demong, N. J. 1970 Flea beetles and mustard oils: host plant specificity of *Phyllotreta cruciferae* and *P. striolata* adults (Coleoptera: Chrysomelidae). *Ann. ent. Soc. Am.* **63**, 832–841.

Gates, D. M. 1980 *Biophysical Ecology*. New York: Springer-Verlag.

Gilbert, L. E. 1982 The coevolution of a butterfly and vine. *Scient. Am.* **247**, 102–107.

Givnish, T. J. 1979 On the adaptive significance of leaf form. In *Topics in plant population biology* (ed. O. T. Solbridge, S. Jain, G. B. Johnson & P. H. Raven), pp. 375–407. New York: Columbia University Press.

Givnish, T. J., Sytsma, K. J., Smith, J. F. & Hahn, W. J. 1994 Thorn-like prickles and heterophylly in Cyanea: adaptations to extinct avian browsers in Hawaii? *Proc. natl. Acad. Sci. U.S.A.* **91**, 2810–2814.

James, R. R., McEvoy, P. B. & Cox, C. S. 1992 Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*), an experimental analysis. *J. appl. Ecol.* **29**, 589–596.

Matsuki, M. & MacLean, S. F. Jr 1994 Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia* **100**, 141–152.

McCullagh, P. & Nelder, J. A. 1983 *Generalised Linear Models*. London: Chapman and Hall.

McKey, D. 1979 The distribution of secondary compounds within plants. In *Herbivores: their interaction with secondary plant metabolites* (ed. G. A. Rosenthal & D. H. Janzen), pp. 55–133. New York: Academic Press.

Meyer, G. A. & Montgomery, M. E. 1987 Relationships between leaf age and the food quality of cottonwood foliage for the gypsy moth, *Lymantria dispar*. *Oecologia* **72**, 527–532.

Mitchell, B. K. 1988 Adult leaf beetles as models for exploring the chemical basis of host plant selection. *J. Insect Physiol.*, **34**, 213–225.

Niemela, P. & Tuomi, J. 1987 Does leaf morphology of some plants mimic caterpillar damage? *Oikos* **50**, 256–257.

Nuñez-Farfán, J. & Dirzo, R. 1994 Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. *Evolution* **48**, 423–436.

Palaniswamy, P. & Bodnaryk, R. P. 1994 A wild brassica from Sicily provides trichome-based resistance against flea beetles, Phyllotreta cruciferae (Goeze) (Coleoptera, Chrysomelidae). *Can. Ent.* **125**, 1011–1021.

Parkhurst, D. F. & Loucks, O. L. 1972 Optimal leaf size in relation to environment. *J. Ecol.* **60**, 505–537.

Peng, C., Weiss, M. J. & Anderson, M. D. 1992 Flea beetle (Coleoptera: Chrysomelidae) response, feeding, and longevity on oilseed rape and crambe. *Environ. Ent.* **21**, 604–609.

Prokopy, R. J. & Owens, E. D. 1983 Visual detection of plants by herbivorous insects. *A. Rev. Ent.* **28**, 337–364.

Raupp, M. J. & Denno, R. J. 1983 Leaf age as a predictor of herbivore distribution and abundance. In *Variable plants and herbivores in natural and managed systems* (ed. R. F. Denno & M. S. McClure), pp. 91–124. New York: Academic Press.

Rausher, M. D. 1978 Search image for leaf shape in a butterfly. *Science, Wash.* **200**, 1071–1073.

Schillinger, J. A. & Gallun, R. L. 1968 Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, *Oulema melanopus*. *Ann. ent. Soc. Am.* **61**, 900–903.

Southwood, R. 1986 Plant surfaces and insects: an overview. In *Insects and the plant surface* (ed. B. Juniper & R. Southwood), pp.1–22. London: Edward Arnold.

Stork, N. E. 1980 Role of waxblooms in preventing attachment to brassicas by the mustard beetle, *Phaedon cochleariae*. *Ent. exp. Appl.* **28**, 100–107.

Talbert, C. M. & Holch, A. E. 1957 A study of the lobing of sun and shade leaves. *Ecology*. **38**, 655–658.

Tanton, M. T. 1962 The effect of leaf 'toughness' in the feeding of larvae of the mustard beetle *Phaedon cochleariae* Fab. *Ent. Exp. Appl.* **5**, 74–78.

Van Lenteren, J. C. & de Ponti, O. M. B. 1990 Plant-leaf morphology, host–plant resistance and biological control. *Symp. Biol. Hung.* **39**, 365–386.

Vaughn, T. T. & Hoy, C. W. 1993 Effects of leaf age, injury, morphology and cultivars on feeding behaviour of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Environ. Ent.* **22**, 418–424.

Vincent, C. & Stewart, R. K. 1984 Effect of allyl isothiocyanate on field behaviour of crucifer-feeding flea beetles (Coleoptera: Chrysomelidae). *J. chem. Ecol.* **10**, 33–39.

Vogel, S. 1970 Convective cooling at low airspeeds and the shapes of broad leaves. *J. exp. Bot.* **21**, 91–101.

Weaver, J. E. & Clements, F. E. 1938 *Plant Ecology*. New York: McGraw-Hill Book Co.

Whitham, T. G. 1983 Individual trees as heterogeneous environments: adaptation to herbivory or epigenetic noise? In *Insect life history patterns: habitat and geographic variation* (ed. R. F. Denno & H. Dingle), pp. 9–27. New York: Springer-Verlag.

Woodhead, S. & Chapman, R. F. 1986 Insect behaviour and the chemistry of plant surface waxes. In *Insects and the plant surface* (ed. B. Juniper & R. Southwood), pp.123–135. London: Edward Arnold.

Woodwell, G. M., Whittaker, R. H. & Houghton, R. A. 1975 Nutrient concentrations in plants in the Brookhaven oak–pine forest. *Ecology* **56**, 318–332.

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