

# Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution?

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**Keywords** *Cylindrotoma distinctissima* · Self-selection · Nutritional ecology · Polyphagy · Plant-insect interaction

**Abstract** We reared larvae of three generalist insect species on plants occurring in their habitats. Individuals of each species were kept either on mixed diets, or on each plant species separately. We measured food plant preference in the mixed-diet group and compared insect performance on single plants to the performance on the mixed diet. For all three insect species, food choice within the mixed-diet groups was non-random and delivered the best overall performance, thus fulfilling the criteria for self-selected diets. When a single diet was as good as the mixed diet for one particular aspect of performance (*Adenostyles alliariae* and *Petasites albus* for *Miramella alpina*; *A. alliariae* for *Callimorpha dominula*), it was never the most preferred food plant in the mixed-diet treatment. Whether the benefit achieved by mixing diets is due to nutrient complementation or toxin dilution, we argue that there is no easy way to distinguish between the two hypotheses on the basis of consumption and performance measurements, as has previously been proposed. From the interpretation of utilisation plots, the ANCOVA equivalent of nutritional indices, we were able to gain insight into where in the sequence from ingestion to growth (preingestive, predigestive or postdigestive) single diets caused differences from mixed diets. The elements of this control system

which were influenced by single diets varied considerably, both within and between insect species. No food plant was toxic or deterrent to all experimental insect species; a food plant that caused consumption effects (preingestive) for one insect species could be dealt with metabolically (postdigestive) by another; different food plants could cause behavioural effects (preingestive), metabolic effects (postdigestive), or a combination of both effects, all within the same insect species. However, one generality did emerge: once a food was ingested, further growth-relevant effects occurred metabolically (postdigestive) rather than via differential egestion (digestibility).

## Introduction

Although capable of exclusive feeding from a wide range of food plants, it has been shown that many generalist invertebrate herbivores perform best when presented with a mixture of food plants rather than a single plant, e.g. butterflies (Merz 1959), grasshoppers (Hodge 1933; Barnes 1955; Freeland 1975; Hoekstra and Beenackers 1976; Chandra and Williams 1983; Bernays et al. 1994) and a sea hare (Pennings et al. 1993). Excluding other possible advantages of a generalist diet, such as savings in food search time or a greater choice in enemy-free space, these experiments explored the importance of the nutritional value of individual foods versus a mixture of foods. In the interpretation of the results, some controversy arose as to whether the primary importance of mixed diets was the maintenance of nutritional balance (Pulliam 1975; Rapport 1980) or the dilution of allelochemicals contained in the food plants (Freeland and Janzen 1974).

Recently Pennings et al. (1993) and Bernays et al. (1994) suggested a relatively straightforward way to estimate the relative importance of both possibilities. The argument is as follows. If it is assumed that animals kept on single diets lack optimal conditions, either due

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to an imbalance of nutrients or to an overdose of toxins, then under both hypotheses one would predict reduced growth for animals kept on single diets compared to animals kept on mixed diets. The crucial difference between the hypotheses lies in their prediction about consumption of the single diets compared to the mixed diet. In the case of the nutrient complementation hypothesis (hereafter NH), reduced growth should accompany an increased consumption of single diets, since the animals are trying to compensate for nutrients that are in low supply. Under the assumption that consumption might be constrained, e.g. by gut size or nutritional imbalances, unchanged consumption would also fit the hypothesis. In the case of the non-interactive toxins hypothesis (hereafter TH), reduced growth should co-occur with reduced consumption of the diet, since the animals have to avoid high doses of whatever toxin is contained in the diet.

Although the reasoning in distinguishing between the NH and the TH seems logical it is not necessarily true. The assumption that mixed diets will always be better than single diets is only adequate when no single diet comes close to the insect's nutrient requirements, the "intake target" (Raubenheimer and Simpson 1993; Simpson and Raubenheimer 1993b), and when alternative foods are available which happen to complement each other in limiting nutrients or toxin content. Likewise, the assumption that under a specific nutrient limitation, food consumption will increase (NH), is ill founded (Raubenheimer 1992). While it is true that the response to limiting nutrients may be to increase consumption until limiting requirements are met, irrespective of whatever other nutrients are ingested in excess (e.g. salt intake under protein and carbohydrate constraints in *Locusta migratoria*; Trumper and Simpson 1993), this need not always be the case. Indeed, a range of situations has been described, in both insects and vertebrates, where the ingestion of excess nutrients constrains overall intake, which would have had to be increased in order to meet requirements for limiting nutrients (Raubenheimer 1992; Simpson and Raubenheimer 1993b, 1996). A possible mechanism for this response has been described by Abisgold and Simpson (1988), showing that high levels of some amino acids in the *L. migratoria* haemolymph will reduce the sensitivity of taste receptors, which ultimately leads to reduced consumption until the excess amino acids are removed. Within the framework of the NH and TH as stated above, this outcome, although clearly due to nutritional imbalances, would be interpreted as a toxin effect.

Given these complications in the interpretation of growth and consumption, it might be impossible to distinguish between the NH and TH with only this information at hand. Since the definition of toxins, as causing performance and consumption reductions, is clearly problematic and may well apply to nutrient limitation as well, it is probably wiser to do without the nutrient/toxin distinction and study their interaction within a more unified context (Simpson et al. 1995).

In our study, we have used current statistical methods (Raubenheimer and Simpson 1994) to investigate the influence of single and mixed diets on food consumption and growth in three generalist insect herbivores, *Miramella alpina* (Acrididae), *Callimorpha dominula* (Arctiidae) and *Cylindrotoma distinctissima* (Tipulidae). These species differ in characteristics such as development, cryptic coloration and mobility, but can all be found feeding on *Adenostyles alliariae* (Asteraceae). Rather than concentrating on potential pest species as model generalists, we aimed to investigate whether generalists which frequently use a specific host plant also benefit from dietary mixing, and whether this pattern would hold across a range of phenologically very different insect species.

If we found insect performance to differ between various diets, we asked whether these differences were due to effects on consumption (preingestive), food utilisation (postingestive) or a combination of both. Having established that there is a postingestive influence of diet on growth, we then asked whether predigestive (differential egestion) or postdigestive (differential metabolic utilisation) mechanisms caused this effect. In the latter case, growth differences between dietary treatments reflect the different proportions of digested mass which have been allocated to growth metabolism versus maintenance metabolism. However, whether a high allocation of digested mass away from growth metabolism reflects higher energy needs for detoxification, excretion (uric acid production) or "wastage respiration" (Zanotto et al. 1993, 1997) remains unresolved.

## Material and methods

### Experimental procedure

We provided leaves from all food plants either simultaneously (mixed diet), or singly to larvae of the three insect species. All larvae were collected in the field and brought to the laboratory where they were weighed and randomly distributed across the experimental treatment groups. The larvae were kept individually in closed plastic containers (9 cm diameter) which had a damp plaster bottom lined with filter paper which was changed whenever necessary. The containers were placed in incubators set at  $17 \pm 2^\circ\text{C}$ , 16 h light. At the beginning and the end of the final larval instar, we weighed the larvae again and determined their consumption and faeces production (dry mass) throughout the instar. Final fresh weight was measured either of newly emerged adults (*M. alpina*) or of pupae (*C. dominula* and *C. distinctissima*).

We prepared food by cutting out leaf disks of experimental plants with a cork borer and set aside reference disks for drying and weighing. We also collected the leftover food, pooled all leftovers from within a treatment of the same larva, dried and weighed them. The intervals between feedings were 2–3 days, whereas we checked for important events, such as moults or a possible food shortage, every day.

### Experimental insects

*M. alpina* Kollar (Caelifera, Acrididae) is a cryptic, highly mobile grasshopper of humid montane areas of central Europe from about 500–2200 m elevation. In Switzerland, it is a characteristic species

of slopes above 1000 m. It is polyphagous on dicotyledonous plants and occasionally causes local outbreaks defoliating trees (Harz 1975). The experimental specimens were collected in their penultimate nymphal stage in the Chasseral range (Swiss Jura mountains) at 1350 m elevation in July 1994. None of the experimental food plants grew close to the collecting site, but we have observed *M. alpina* feeding on most experimental food plants elsewhere.

Caterpillars of *C. dominula* L. (Lepidoptera, Arctiidae) are aposematically coloured and highly mobile in the field. They are reported to feed on at least 48 plant species of humid areas belonging to 28 different plant families (Carter and Hargreaves 1987; Owen 1994). The experimental specimens originated from the northern slope of the Blauen (Swiss Jura mountains) at 800 m elevation and were collected in May 1993 in their penultimate larval stage. We collected most caterpillars from leaf litter near *A. alliariae*, *Fagus sylvatica* seedlings and *Senecio fuchsii*; on various other occasions we observed caterpillars feeding from these plants.

*C. distinctissima* Meigen (Diptera, Tipulidae) is cryptic and of low mobility in its larval stage. To oviposit, the females cut the lower epidermis of a leaf with a saw formed by the two valvae of their ovipositor. Therefore, the choice of larval food plants is made by the adult female. Sixteen plant species from seven families have been recorded as food plants (Peus 1952; Brodo 1967; Rotheray 1989). We collected all experimental specimens at the beginning of their ultimate larval stage from Zastler (Black Forest, Germany) at 1100 m elevation in May 1993. We collected half of the larvae from leaves of *A. alliariae* and the other half from *Petasites albus* (Asteraceae).

#### Experimental food plants

Table 1 lists the experimental food plants together with their growth characteristics and dominant plant secondary compounds. Plant taxonomy, range and ecological information is given according to Oberdorfer (1983). We chose these plants because they were among the most abundant herbaceous plants at the Blauen location and because they were all acceptable to *C. dominula*, the herbivore we collected from the same location. We collected

*P. albus* at Lieserwasen, a locality in the Vosges (France) at 600 m altitude, and all other plants at the Blauen location. All food plants were collected weekly, put in plastic bags, transported in a cooled box and stored in a refrigerator at 4°C until used. So each week, the first feeding was on freshly collected material, the second and third feeding on 2- and 4-day-stored plant material. Foliage collected under similar conditions was shown to have stable protein and phenolic compound content for up to 2 days when kept hydrated under insect-rearing temperature conditions. From 3 to 4 days, protein and total phenolic levels were elevated reaching initial levels again 5 days after collection (Kleiner 1991). Although we do not know the stability of nutrients and allelochemicals in our leaf disk preparations, the standardised handling of our plant material assures that whatever changes might have taken place in the leaf disks were equally experienced by all our insect species. Recent evidence also suggests low turnover of stored allelochemicals, including pyrrolizidine alkaloids (Mihaliak et al. 1991; Hartmann 1996).

#### Data analysis

To compare the performance of the larvae between the different treatment groups, we calculated analyses of variance (ANOVAs) and analyses of covariance (ANCOVAs) where appropriate. Since the analyses of nutritional ratios (Waldbauer 1968) have been criticised for different reasons by various authors (Schmidt and Reese 1986; Raubenheimer and Simpson 1992, 1994; Horton and Redak 1993; van Loon 1993; Raubenheimer 1995), we performed ANCOVAs to investigate growth and faeces production in the different experimental treatments. We thereby maintained the logic of Waldbauer's (1968) indices without committing the severe potential statistical errors associated with the index analyses. The ANCOVA equivalent to Waldbauer's (1968) "efficiency of conversion of ingested food to body substance (ECI)" is to compare growth between the different dietary treatments taking consumption as the covariate of the analysis. As outlined by Raubenheimer and Simpson (1992, 1994) and Horton and Redak (1993), this analysis also allows us to distinguish whether effects on growth are

**Table 1** Experimental food plants, ecological characteristics and secondary leaf compounds (*PA* pyrrolizidine alkaloid)

Food plant	Description	Insect feeders (likely insect feeders)	Known secondary compounds in leaves	References
<i>Adenostyles alliariae</i> (Asteraceae)	Alpine perennial on nutrient-rich soils	<i>Callimorpha dominula</i> , <i>Cylindrotoma distinctissima</i> , <i>Miramella alpina</i>	Seneciphylline and other PAs (sesquiterpenes of furoeremophilane type <sup>a</sup> )	Harmatha et al. (1969); Seaman (1982); Rowell-Rahier et al. (1991)
<i>Senecio fuchsii</i> (Asteraceae)	Alpine perennial on nutrient-rich soils	<i>C. dominula</i> , <i>C. distinctissima</i> ( <i>M. alpina</i> )	Retronecine, platyphylline and other PAs, sesquiterpenes of furoeremophilane type	Seaman (1982); Rowell-Rahier et al. (1991); J. Harmatha (personal communication)
<i>Petasites albus</i> (Asteraceae)	Montane perennial on nutrient-rich soils	<i>C. dominula</i> , <i>C. distinctissima</i> ( <i>M. alpina</i> )	Sesquiterpenes of furoeremophilane type <sup>b</sup>	Novotny et al. (1964); Seaman (1982)
<i>Urtica dioica</i> (Urticaceae)	Perennial on humid nutrient-rich soils	<i>C. dominula</i> ( <i>C. distinctissima</i> , <i>M. alpina</i> )	Acetylcholine, histamine, 5-hydroxytryptamine (nettle hairs) quercetine, <i>p</i> -coumaric acid, nicotine (leaves)	Hegnauer (1973)
<i>Lamium galeobdolon</i> (Lamiaceae)	Forest perennial on nutrient-rich soils	<i>C. dominula</i> ( <i>C. distinctissima</i> , <i>M. alpina</i> )	Tannins, flavonolglycosides, caffeic acid <sup>c</sup>	Hegnauer (1966)
<i>Fagus sylvatica</i> (Fagaceae)	Dominant tree species, only cotyledons used	<i>C. dominula</i> ( <i>M. alpina</i> )	Glycosides of methylsalicylic acid	Tailleur (1901)

<sup>a</sup> Sesquiterpenes only in trace amounts in leaves, but abundant in rhizomes

<sup>b</sup> Sesquiterpene content of leaves is not exactly known, but presence is likely

<sup>c</sup> All described from closely related *Lamium* species

caused pre- or postingestively. Preingestive effects on growth are caused by a proportionately lower or higher consumption which is shown in the ANCOVA plot by data points which are an extrapolation of the control group data points thus extending the fitted data line of the control group in either direction. Postingestive effects of treatments are independent of consumption and therefore shown in the ANCOVA plot as data points lying on a line parallel to the data points of the control group. Mechanisms occurring after ingestion but prior to digestion (differential egestion, food digestibility) are detected by comparing the amounts of faeces produced under the different dietary treatments taking consumption as a covariate. This is the ANCOVA equivalent of Waldbauer's (1968) nutritional index "approximate digestibility (AD)". Postdigestive mechanisms are detected by comparing growth between dietary treatments taking digested mass (intake minus faeces) as covariate. This analysis is the ANCOVA equivalent of Waldbauer's (1968) "efficiency with which digested food is converted to body substance (ECD)". The interpretation of the ANCOVA plots of ECD allows conclusions about pre- and postdigestive effects on growth in a similar way as explained above for pre- and postingestive effects.

As outlined by Sokal and Rohlf (1995), we used the treatment $\times$ covariate interaction term to test for homogeneity of regression slopes and when we found no significant difference between slopes, we proceeded with testing the adjusted group means. In cases of a divergence of regression slopes, we tested for differences in slopes between the mixed-diet group and single-diet groups, as well as for differences in mean values at the centre of accuracy (Maxwell and Delaney 1990). For pairwise comparisons, we used Bonferroni-adjusted *P*-values to account for multiple testing. We performed all ANOVAs and ANCOVAs using type III sum of squares which tolerate unbalanced cell sizes. For post hoc testing of significant results, we used Dunnett's test set at  $P < 0.05$  to see whether any single-diet group was different from the mixed-diet group.

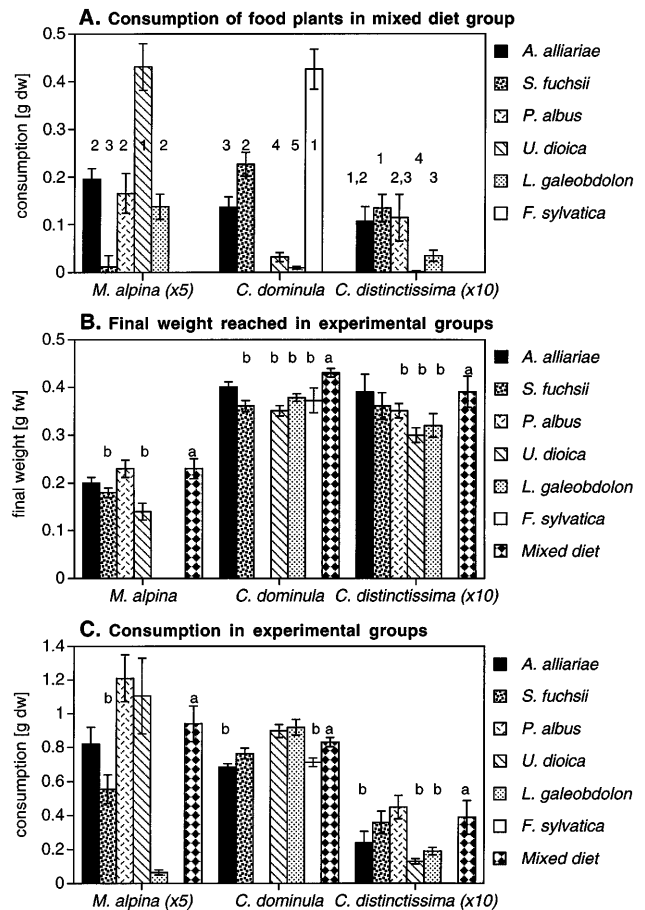
In *C. distinctissima*, females and males differed considerably in their pupal weight, therefore we performed two-way ANOVAs and ANCOVAs using "sex" as the second factor. The influence of the dietary regime ("diet") was regarded as a random factor and the sex of the individuals ("sex") as a fixed factor.

Within the mixed-diet experimental group, we tested for food preferences using the Friedman test as suggested by Roa (1992). For post hoc testing between dietary components, we used the formula given by Conover (1981). All analyses were computed with the program SuperAnova from Abacus (1989) on a Macintosh computer.

## Results

### Food plant preferences

As shown in Fig. 1A, all species showed a clear preference hierarchy for the food plants offered in the mixed-diet treatment (*M. alpina*:  $df$  4,  $\chi^2$  33.16,  $P < 0.001$ ; *C. dominula*:  $df$  4,  $\chi^2$  42.07,  $P < 0.001$ ; *C. distinctissima*:  $df$  5,  $\chi^2$  27.65,  $P < 0.001$ ). *M. alpina* preferred *Urtica dioica* over all other food and *A. alliarieae*, *P. albus* and *Lamium galeobdolon* were preferred over *S. fuchsii*. *C. dominula* preferences were ranked: *F. sylvatica* > *S. fuchsii* > *A. alliarieae* > *U. dioica* > *L. galeobdolon*. *C. distinctissima* showed a grouping of preferences, preferring *S. fuchsii* to all other plants except *A. alliarieae*; *A. alliarieae* was preferred to *L. galeobdolon* but not to *P. albus*, and *U. dioica* was the least chosen food. *F. sylvatica* was not eaten by this species and was therefore omitted from the analysis.



**Fig. 1** A Consumption of food plants (preferences) of *Miramella alpina* (Acrididae), *Callimorpha dominula* (Arctidae) and *Cylindrotoma distinctissima* (Tipulidae) within the mixed-diet treatments. Mean dry weights and SEs are shown. Values of *M. alpina* are multiplied by 5, those of *C. distinctissima* by 10. Significant differences between groups are indicated by different numbers above the columns ( $P < 0.05$ ). B Final weight of larvae under the different diet regimes. Mean fresh weight and SEs are shown. Values of *C. distinctissima* are multiplied by 10. Letters above columns indicate significant differences from the mixed-diet group ( $P < 0.05$ ). C Total consumption of the different diets by larvae during the experimental period. Mean dry weight and SEs are shown. Values of *M. alpina* are multiplied by 5, those of *C. distinctissima* by 10. Letters above columns indicate significant differences from the mixed-diet group ( $P < 0.05$ ).

### Performance on mixed and single diets

#### *M. alpina*

All animals which were kept only on *L. galeobdolon* died after about 4 days, probably due to poisoning, since they had all eaten from the leaf disks. Performance, measured as weight at the final moult, differed between the experimental diet groups (Fig. 1B, Tables 2, 3). Nymphs raised on *S. fuchsii* and *U. dioica* reached a significantly lower weight than those raised on the mixed diet, whereas there was no difference between mixed-diet- and *A. alliarieae*- and *P. albus*-reared nymphs.

Consumption on *S. fuchsii* was lower than that of the mixed-diet group (Fig. 1C, Tables 2, 3). Less faeces were

**Table 2** Measures and results of analyses of covariance of last-instar *Miramella alpina* kept on single and mixed diets. Mean values are shown. All measures were tested for differences between experimental groups by ANOVA. Means of single-diet values fol-

lowed by a letter are significantly different from the mixed-diet experimental group, as revealed by Dunnett's post hoc test ( $P < 0.05$ ). For ANOVAs and ANCOVAs see Table 3

Measure	P-value	Last nymphal stage kept on					
		<i>Adenostyles alliariae</i>	<i>Senecio fuchsii</i>	<i>Petasites albus</i>	<i>Urtica dioica</i>	<i>Lamium galeobdolon</i>	Mixed diet
Initial weight (g fresh weight)	0.0706	0.096	0.093	0.118	0.091	(0.099)	0.109
Final weight (g fresh weight)	0.0267	0.208	0.180 b	0.233	0.143 b		0.231 a
Consumption (g dry weight)	0.0037	0.164	0.111 b	0.242	0.221	(0.013)	0.188 a
Faeces produced (g dry weight)	0.0014	0.108 c	0.098 c	0.196 a	0.135	(0.014)	0.150 b
Food assimilated (g dry weight)	0.0212	0.056	0.013	0.046	0.086		0.038
Last-instar duration (days)	0.1084	17.6	20.1	20.7	21.1	(4.1)	17.1
ANCOVA ECI	0.0003				b		a
ANCOVA AD	0.0020	c	c	a			b
ANCOVA ECD	0.0034				b		a
Number of insects		12	12	9	9	(12)	13

**Table 3** Results of ANOVAs and ANCOVAs of performance measures of *M. alpina*. (a)–(e): tests for differences in regression slopes (diet  $\times$  covariate interaction) were not significant (a:  $P = 0.3878$ ; b:  $P = 0.8858$ ; c:  $P = 0.1827$ ; d:  $P = 0.7699$ ; e:  $P = 0.6020$ )

Type of analysis	Source of variation	df	MS	F	P
ANOVA					
Initial weight	Diet	5	0.0012	2.157	0.0706
	Residual	61	0.0005		
Faeces produced	Diet	4	0.0153	5.211	0.0014
	Residual	50	0.0029		
Food assimilated	Diet	4	0.0074	3.172	0.0212
	Residual	50	0.0023		
Last-instar duration	Diet	4	37.725	2.003	0.1084
	Residual	50	18.833		
ANCOVA					
(a) Final weight	Diet	4	0.0067	3.012	0.0267
	Covariate: initial weight	1	0.0397	17.746	0.0001
	Residual	49	0.0022		
(b) Consumption	Diet	4	0.0262	4.461	0.0037
	Covariate: initial weight	1	0.0742	12.648	0.0008
	Residual	49	0.0058		
(c) Weight gain (ECI)	Diet	4	0.0069	6.436	0.0003
	Covariate: consumption	1	0.0346	32.295	0.0001
	Residual	49	0.0011		
(d) Faeces (AD)	Diet	4	0.0041	4.941	0.0020
	Covariate: consumption	1	0.1055	126.238	0.0001
	Residual	49	0.0008		
(e) Weight gain (ECD)	Diet	4	0.0072	4.531	0.0034
	Covariate: food assimilated	1	0.0082	5.107	0.0283
	Residual	49	0.0016		

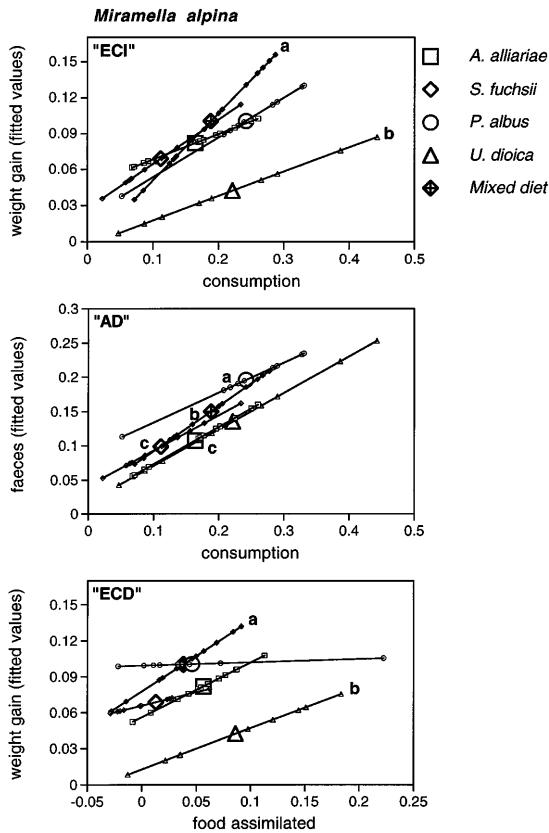
produced by the nymphs kept on *A. alliariae* and *S. fuchsii* and more by those kept on *P. albus* compared to the animals kept on the mixed diet. The dry mass of assimilated food differed between the *S. fuchsii* and the *U. dioica* group, but neither differed from the mixed-diet group.

The plots of the ANCOVA analyses ECI, AD and ECD (Fig. 2) revealed at which level, from consumption to growth, single diets differed from the mixed diet in their effects on growth. The ECI analysis shows that from all single diets, only the *U. dioica*-feeding group was unable to convert ingested food into growth comparable to the mixed-diet group, whereas the *S. fuchsii* group did well in converting ingested food into growth. There was no difference in digestibility between *U. dioica* and the mixed diet (Fig. 2B) whereas a marked differ-

ence was apparent in the capability to convert assimilated food into growth (Fig. 2C). Digestive efficiencies in the groups kept on *A. alliariae*, *S. fuchsii* and *P. albus* were different from the mixed-diet group, although without having marked influences on performance (Fig. 2B, Table 2).

### *C. dominula*

Performance, measured as pupal weight, differed between experimental groups (Fig. 1B, Tables 4, 5). All caterpillars which were kept on single diets, except those kept on *A. alliariae*, reached significantly lower pupal weights than the caterpillars kept on the mixed diet. The duration of the last instar was prolonged in the groups



**Fig. 2** Bicoordinate plots of ANCOVAs on weight gain and faeces production of *M. alpina*. The plot of weight gain against consumption, *ECI* (A), corresponds to Waldbauer's (1968) "efficiency of conversion of ingested food" which visualises how much of the consumed food was allocated to growth. The faeces/consumption plot, *AD* (B), corresponds to the "approximate digestibility", a measure of the digestibility of a particular food. The weight gain/assimilated food plot, *ECD* (C), corresponds to the "efficiency of conversion of digested food" which visualises how much of the digested food was allocated to growth. *y*-values are fitted by ANCOVA (see Table 3). Large symbols display the group means, while small symbols indicate the individual values. Letters indicate significant differences between single-diet groups and the mixed-diet group

kept on *S. fuchsii* and *U. dioica* compared to the mixed-diet group.

Caterpillars kept on *A. alliariae* and *F. sylvatica* consumed less leaf mass than those kept on the mixed diet (Fig. 1C, Tables 4, 5). Caterpillars which were kept on *S. fuchsii* produced less faeces than those kept on the mixed diet. The dry mass of assimilated food in single-diet groups, compared to the mixed-diet group, was lower in groups kept on *F. sylvatica* and *A. alliariae* and higher in groups kept on *U. dioica* and *S. fuchsii*.

The plot of weight gain against consumption (Fig. 3A) revealed significantly different regression slopes for the different experimental groups (Table 5). The *U. dioica*-fed group was the only group showing a different regression slope from the mixed-diet group ( $t = 2.106$ ,  $P = 0.0396$ ), which indicates strong post-ingestive effects of this diet. The *F. sylvatica*-fed group stands out as the only one showing a negative regression

slope as well as a reduction in consumption. If the dataset is analysed without the *F. sylvatica* group, the regression slopes converge to parallelism and the *L. galeobdolon*-, *S. fuchsii*- and *U. dioica*-fed groups stand apart from the *A. alliariae* and the mixed-diet group. Testing for differences between the mixed-diet group and single-diet groups at the centre of accuracy of covariate values (Maxwell and Delaney 1990) revealed that all groups except *A. alliariae* had lower weight gains per mean amount of ingested food.

All post-ingestive diet effects on growth were caused postdigestively, as the digestibility (AD) and ECD plots show (Fig. 3). Whereas there were now differences in digestibility between the mixed diet and most single diets, the digestibility of *S. fuchsii* was even enhanced. However, enhanced digestibility was not translated to increased growth on this diet. Instead the ECD plot mirrors closely the ECI analysis which indicates that differential digestibility had little or no effect on growth performance.

### *C. distinctissima*

The performance (pupal weight) of larvae raised on *P. albus*, *U. dioica* or *L. galeobdolon* alone was significantly decreased compared to that of larvae raised on the mixed diet (Fig. 1B, Tables 6, 7). The duration until pupation was prolonged on all single diets, except for *P. albus*, compared to the mixed diet. The larvae kept on *F. sylvatica* did not feed from it.

The consumption of *A. alliariae*, *U. dioica* and *L. galeobdolon* was decreased compared to the consumption of the mixed diet (Fig. 1C, Tables 6, 7). Faeces production did not differ between the experimental groups, but less food was assimilated by the larvae fed on *A. alliariae*, *U. dioica* and *L. galeobdolon* than by those fed the mixed diet.

The ANCOVA analysis of the plot of weight gain against consumption (Fig. 4A, Table 6) reveals that only *U. dioica*- and *P. albus*-fed larvae showed reduced growth compared to the mixed-diet group. Decreased consumption, and even decreased digestibility, of *A. alliariae* (Fig. 4B) had no effect on growth performance of the larvae. After intake, the effects of the *P. albus* diet on growth were exclusively due to post-ingestive mechanisms (no differences in digestibility, reduced weight gain per unit assimilated food, Fig. 4B,C). Post-ingestive effects of the *U. dioica* diet, however, were twofold. Decreased digestibility and post-ingestive effects both contributed to the reduced growth performance of *U. dioica*-fed larvae (Fig. 4B,C).

## Discussion

In the discussion of our results we will initially focus on the specific interaction between each herbivore and food plant species. We will try to relate information about

**Table 4** Measures and results of analyses of covariance of last-instar *C. dominula* kept on single and mixed diets. Mean values are shown. All measures were tested for differences between experimental groups by ANOVA. Means of single-diet values followed by a letter are significantly different from the mixed-diet experi-

mental group, as revealed by Dunnett's post hoc test ( $P < 0.05$ ) [*n.a.* not applicable since regression slopes are different; letters in parentheses refer to differences as revealed by pairwise comparisons with the ANCOHET model (Maxwell and Delaney 1990)]. For ANOVAs and ANCOVAs see Table 5

Measure	P-value	Larvae kept on					
		<i>Adenostyles alliariae</i>	<i>Senecio fuchsii</i>	<i>Urtica dioica</i>	<i>Lamium galeobdolon</i>	<i>Fagus sylvatica</i>	Mixed diet
Initial weight (g fresh weight)	0.3936	0.162	0.164	0.175	0.182	0.186	0.189
Pupal weight (g fresh weight)	0.0001	0.408	0.363 b	0.352 b	0.378 b	0.372 b	0.430 a
Consumption (g dry weight)	0.0001	0.684 b	0.763	0.897	0.918	0.713 b	0.831 a
Faeces produced (g dry weight)	0.0001	0.537	0.383 b	0.556	0.623	0.578	0.593 a
Food assimilated (g dry weight)	0.0001	0.147 c	0.380 a	0.341 a	0.295	0.135 c	0.238 b
Last-instar duration (days)	0.0001	27.5	28.1 a	29.5	27.7	24.9	26.0 b
ANCOVA ECI	<i>n.a.</i>		(b)	(b)	(b)	(b)	(a)
ANCOVA AD	0.0001		b				a
ANCOVA ECD	<i>n.a.</i>		(b)	(b)	(b)	(b)	(a)
Number of insects		12	11	11	12	12	12

**Table 5** Results of ANOVAs and ANCOVAs of performance measures of *C. dominula*. (a)–(c): tests for differences in regression slopes (diet × covariate interaction) were not significant (a:  $P = 0.9671$ ; b:  $P = 0.0522$ ; c:  $P = 0.9013$ )

Type of analysis	Source of variation	df	MS	F	P
ANOVA					
Initial weight	Diet	5	0.0016	1.055	0.3936
	Residual	64	0.0015		
Faeces produced	Diet	5	0.0806	11.468	0.0001
	Residual	64	0.0070		
Food assimilated	Diet	5	0.1174	15.327	0.0001
	Residual	64	0.0077		
Last-instar duration	Diet	5	29.939	8.336	0.0001
	Residual	64	3.589		
ANCOVA					
(a) Pupal weight	Diet	5	0.0096	11.508	0.0001
	Covariate: initial weight	1	0.0238	28.630	0.0001
	Residual	63	0.0008		
(b) Consumption	Diet	5	0.0993	9.786	0.0001
	Covariate: initial weight	1	0.1678	16.545	0.0001
	Residual	63	0.0101		
Weight gain (ECI)	Diet	5	0.0078	7.922	
	Covariate: consumption	1	0.0034	3.440	
	Diet × covariate	5	0.0025	2.584	0.0354
	Residual	58	0.0010		
(c) Faeces (AD)	Diet	5	0.0689	16.004	0.0001
	Covariate: consumption	1	0.1691	39.307	0.0001
	Residual	63	0.0043		
Weight gain (ECD)	Diet	5	0.0047	4.536	
	Covariate: food assimilated	1	0.0001	0.002	
	Diet × covariate	5	0.0028	2.698	0.0293
	Residual	58	0.0010		

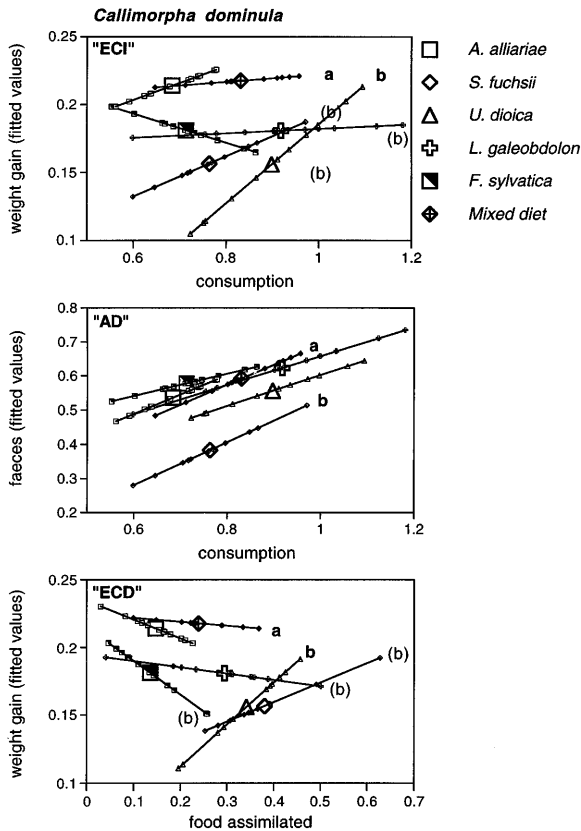
relatedness of plant species, allelochemical and nutrient content to the actual choice and performance of the specific herbivore. Finally, we discuss general issues raised by our results and what their implication might be for principles of the feeding ecology of generalist herbivores.

### *M. alpina*

Two of three senecionean food plants, *A. alliariae* and *P. albus*, were as good at providing all necessary nutrients as the mixed diet, since we could not detect any adverse performance effects when they were supplied as single diets. Both plants ranked second in the choice of

the mixed-diet group, which indicates that no strong feeding deterrents were present. In the case of *A. alliariae*, where we know that considerable amounts of pyrrolizidine alkaloids (PAs) are present in leaves (Hägele 1996; Hägele and Rowell-Rahier, in press) and that the nymphs had no opportunity to adjust to *A. alliariae* in the field, this result is somewhat surprising. However, a similar result is reported by Johnson and Bentley (1988) who found no change in short-term growth and dietary efficiency in fifth-instar *Spodoptera eridania* (Lepidoptera, Noctuidae) caterpillars when sparteine or lupanine (two lupine alkaloids) were incorporated into artificial diets. First-instar caterpillars, however, suffered increased mortality on alkaloid-con-

taining artificial diets. Thus, although it seems that allelochemicals can be handled by late instars very effectively (Ehmke et al. 1989), they still might have profound adverse effects on early instars.



**Fig. 3A–C** Bicoordinate plots of ANCOVAs on weight gain and faeces production of *C. dominula*. For definitions of *ECI*, *AD*, *ECD* and symbols see legend to Fig. 2. *y*-values are fitted by ANCOVA (see Table 5). **A, C** Bold letters indicate significant differences in regression slopes, letters in parentheses indicate differences in pairwise comparisons (single groups with mixed-diet group) at their centre of accuracy

**Table 6** Measures and results of analyses of covariance of last-instar *C. distinctissima* kept on single and mixed diets. Mean values are shown. All measures were tested for differences between experimental groups and sex by ANOVA. Means of single-diet values followed by a letter are significantly different from the mixed-diet

Measure	<i>P</i> -value	Larvae kept on					
		<i>Adenostyles alliariae</i>	<i>Senecio fuchsii</i>	<i>Petasites albus</i>	<i>Urtica dioica</i>	<i>Lamium galeobdolon</i>	Mixed diet
Initial weight (g fresh weight)	0.1374	0.026	0.024	0.032	0.033	0.026	0.026
Pupal weight (g fresh weight)	0.0001	0.039	0.036	0.035 b	0.031 b	0.032 b	0.039 a
Consumption (mg dry weight)	0.0007	24.3 b	36.3	45.1	13.1 b	18.9 b	39.2 a
Faeces produced (mg dry weight)	0.1283	11.0	9.2	12.0	9.0	5.9	9.1
Food assimilated (mg dry weight)	0.0001	13.2 b	26.9	33.1	4.0 b	13.0 b	30.1 a
Days until pupation	0.0001	20.1 a	21.8 a	17.8	24.8 a	19.4 a	15.6 b
ANCOVA <i>ECI</i>	0.0002			b	b		a
ANCOVA <i>AD</i>	n.a.	(a)			(a)		(b)
ANCOVA <i>ECD</i>	0.0002			b	b		a
Number of insects		8	12	11	9	10	9

The third senecionean food plant, *S. fuchsii*, caused marked performance effects when offered as a single diet. This was mainly caused by a reduction in consumption of the single diet, corroborated by the low preference for *S. fuchsii* in the mixed-diet treatment. These results support the ideas that a strong deterrent or a lack or imbalance of phagostimulants (nutrients) keeps *M. alpina* from eating *S. fuchsii* in a choice situation and limits its consumption considerably in a no-choice situation. Associative aversion learning, whereby the nymphs were associating a taste component with the poor qualities of the food plant, could also be a mechanism involved in the reduction of consumption, and thus ultimately growth (Lee and Bernays 1988).

Although *U. dioica* was by far the most preferred food in the mixed-diet group, it conferred only poor performance when fed as a single diet. This was due to the inability to convert the digested mass into growth. Increased metabolic activity due to allelochemical processing could be a mechanism causing this effect. A similar result, reduced growth and ECD with no effects on consumption and digestibility, was obtained by Lincoln et al. (1982) when they incorporated plant resins into artificial diets of a given protein composition and fed them to caterpillars of *Euphydryas chalcedona* (Lepidoptera, Nymphalidae). Berenbaum and Zangerl (1994) also found good evidence for the hypothesis that detoxification of the furanocoumarin xanthotoxin imposes a metabolic load on the parsnip webworm, *Depressaria pastinacella* (Lepidoptera, Oecophoridae). However, Neal (1987) was unable to find metabolic costs of mixed-function oxidase induction, which is a metabolic system responsible for most detoxification processes. Furthermore, the processing of excess nutrients (“wastage respiration” and excretion; Zanotto et al. 1993, 1997) could account for increased metabolic activity as a consequence of ingested imbalanced nutrients. Thus it is just as likely that nutritional imbalances impose additional postingestive costs, as it is that these costs are imposed by detoxification mechanisms.

experimental group, as revealed by Dunnett’s post hoc test ( $P < 0.05$ ). [n.a. not applicable since regression slopes are different; letters in parentheses refer to differences as revealed by pairwise comparisons with the ANCOHET model (Maxwell and Delaney 1990)]. For ANOVAs and ANCOVAs see Table 7



**Table 7** Results of ANOVAs and ANCOVAs of performance measures of *C. distinctissima*. (a)–(d): tests for differences in regression slopes (diet × covariate interaction) were not significant (a:  $P = 0.0797$ ; b:  $P = 0.3594$ ; c:  $P = 0.0544$ ; d:  $E5 > P = 0.0888$ ); regression slopes sex × covariate were not significantly different (a:  $P = 0.3580$ ; b:  $P = 0.4000$ ); the interaction term diet × sex × covariate (a:  $P = 0.5258$ ; b:  $P = 0.9659$ ) was omitted from the models

Type of analysis	Source of variation	df	MS	F	P
<b>ANOVA</b>					
Initial weight	Diet	5	0.084	1.770	0.1374
	Sex	1	0.512	6.816	0.0476
	Diet × sex	5	0.075	1.581	0.1838
	Residual	47	0.047		
Faeces produced	Diet	5	0.0185	1.814	0.1283
	Sex	1	1.3913	30.691	0.0026
	Diet × sex	5	0.0453	4.443	0.0021
	Residual	47	0.0102		
Food assimilated	Diet	5	0.8719	7.921	0.0001
	Sex	1	3.5071	9.524	0.0273
	Diet × sex	5	0.3682	3.345	0.0115
	Residual	47	0.1100		
Days until pupation	Diet	5	109.376	9.567	0.0001
	Sex	1	278.368	19.582	0.0069
	Diet × sex	5	14.214	1.243	0.3042
	Residual	47	11.432		
<b>ANCOVA</b>					
(a) Pupal weight	Diet	5	0.1192	12.470	0.0001
	Sex	1	1.7749	26.216	0.0037
	Covariate: initial weight	1	0.0947	9.912	0.0029
	Diet × sex	5	0.0677	7.085	0.0001
	Residual	46	0.0096		
(b) Consumption	Diet	5	0.6787	5.191	0.0007
	Sex	1	10.5831	30.946	0.0026
	Covariate: initial weight	1	1.3416	10.260	0.0025
	Diet × sex	5	0.3420	2.615	0.0366
	Residual	46	0.1308		
(c) Weight gain (ECI)	Diet	5	0.3084	8.587	0.0001
	Covariate: consumption	1	2.0165	56.142	0.0001
	Residual	52	0.0359		
Faeces (AD)	Diet	5	0.0432	5.546	
	Covariate: consumption	1	1.7814	22.867	
	Diet × covariate	5	0.0217	2.789	0.0275
	Residual	47	0.0078		
(d) Weight gain (ECD)	Diet	5	0.3005	6.723	0.0001
	Covariate: food assimilated	1	1.5597	34.891	0.0001
	Residual	52	0.0447		

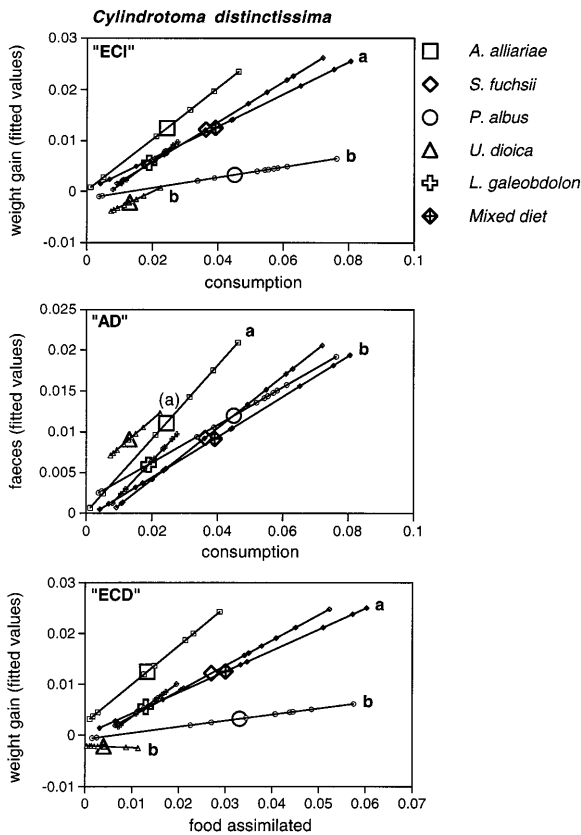
*L. galeobdolon* proved to be toxic to the nymphs when eaten as a single diet. In the mixed-diet group, however, *L. galeobdolon* ranked second in preference and was eaten in much larger quantities than the lethal dose of the single-diet group (Fig. 1). This result supports the idea that within the mixed-diet group, the nymphs were able to dilute the toxic effects of the food and subsequently tolerate it in much higher quantities (Freeland and Janzen 1974).

### *C. dominula*

Performance of *C. dominula* on the two senecionean food plants *A. alliariae* and *S. fuchsii* was remarkably different. Whereas there were no adverse effects associated with the consumption of *A. alliariae* as a single diet, the consumption of *S. fuchsii* caused lower pupal weights and prolonged the developmental period. *A. alliariae* must therefore provide an almost optimally balanced diet for the caterpillars, since they could gain the same amount of weight as in the mixed-diet treatment at an even lower overall consumption. Induced

physiological adaptations might have played a role, since most caterpillars had previously eaten *A. alliariae* in the field. PAs in the food plant do not seem to affect the caterpillars negatively and when given a choice between leaf disks treated with seneciophylline (the main PA of *A. alliariae*), the caterpillars even preferred the treated disks over controls (Hägele 1996). Since the larvae are aposematic they might even exploit PAs for their own defence. Because the caterpillars did so well on the PA-containing *A. alliariae*, PAs are unlikely to have caused the marked postdigestive performance effects associated with the *S. fuchsii* diet. However, allelochemicals could still be responsible for this effect, since *S. fuchsii* contains a different array of PAs as well as sesquiterpenes (Rowell-Rahier et al. 1991). In addition, postdigestive metabolic effects, associated with nutrients (excretion, “wastage respiration”), could account for the observed poor performance.

As in the *S. fuchsii* treatment, caterpillars performed badly when kept on *U. dioica* and *L. galeobdolon* alone. Again the effect was caused postdigestively and must be due to nutritional balancing, detoxification or both. *L. galeobdolon* had no immediate toxic effect on



**Fig. 4A–C** Bicoordinate plots of ANCOVAs on weight gain and faeces production of *C. distinctissima*. For definitions of *ECI*, *AD*, *ECD* and symbols see legend to Fig. 2. *y*-values are fitted by ANCOVA (see Table 7). **B** Bold letters indicate significant differences in regression slopes, letter in parentheses indicates difference in pairwise comparison (single group with mixed-diet group) at their centre of accuracy

*C. dominula*, possibly because the toxic factor was effectively counteracted by the caterpillar's detoxification mechanisms.

*F. sylvatica*, which was the most preferred food in the mixed-diet treatment, supported growth only poorly. The high attractiveness of the cotyledons of *F. sylvatica*, indicated by the preference in the mixed-diet group, may be based in the phagostimulatory power of high levels of reserve proteins and lipids. Postdigestive effects of the diet might reflect the costs associated with the detoxification of substances which are digested along with the nutrients or, perhaps more likely, the cost of dealing with an unbalanced amino acid composition, as frequently found in reserve proteins (Richter 1988). High levels of amino acids in the haemolymph can have a direct influence on consumption by decreasing the sensitivity of amino-acid-sensitive sensilla on maxillary palps as described for *Locusta migratoria* (L.) (Abisgold and Simpson 1988). This mechanism could have been responsible for the decreased consumption when *F. sylvatica* was the single diet. In his experiments with fifth-instar *Celerio euphorbia* (Lepidoptera, Spingidae), House (1969) created a nutritional imbalance (decreased

vitamin, increased casein and selected amino acids, constant sugar and salts) and found a decrease in growth, consumption and *ECI*, similar to our result with *F. sylvatica*.

### *C. distinctissima*

Performance of *C. distinctissima* on the three senecionean food plants (*A. alliariae*, *S. fuchsii* and *P. albus*) differed from that of *M. alpina* and *C. dominula*. Whereas *A. alliariae* and *S. fuchsii* had no adverse effects on the achieved pupal weight, but at the cost of prolonged development, *P. albus*-fed larvae reached lower pupal weights, with no increase in developmental period. There was no difference between the *S. fuchsii* and the mixed diet in digestibility measures, so we conclude that it is as good for growth as the mixed diet, apart from the disadvantage of the longer developmental period. The same is true for *A. alliariae*, where good growth performance is achieved even with decreased digestibility of the diet. It is thus unlikely that PAs imposed significant detoxification costs on the larvae, since among the single diets, the two plants containing PAs supported the best growth. With *P. albus*, the performance costs were clearly due to postdigestive effects on growth. This result is again similar to the findings of Lincoln et al. (1982) when they added plant resin to artificial diets of *E. chalcadon* (Lepidoptera, Nymphalidae).

*U. dioica* was avoided in the mixed-diet group and supported only poor growth as a single diet. This was due to a combination of (preingestive) consumption effects, digestibility effects and postdigestive metabolic effects. At first glance this outcome seems to fit the TH; but again, nutritional imbalances could bring about the same result (see above and House 1969). Postdigestive detoxification as well as uric acid production and wastage respiration could all have tied up energy from digested material and diverted it from growth.

*L. galeobdolon*, which ranked third in preference in the mixed-diet group, also supported only poor growth as a single diet. As our analyses show, this was due entirely to (preingestive) consumption reduction. The presence of a strong feeding deterrent (allelochemical) or a lack or imbalance of phagostimulants (most likely nutrients) could account for strong preingestive effects.

### General discussion and implications

Taking both measured performance criteria into account, mixing proved to be the most successful strategy for all species. Whether self-selection (non-random beneficial shift between foods; see Waldbauer and Friedman 1991), as shown in our experiment, is also an important feature in the field should depend to a high degree on the mobility of the animals. Thus we would expect a decline in self-selection in the field from *M. alpina*, the most mobile of our species, to *C. distinctissima*, which is most likely a facultative monophagous species,

since the larvae probably never leave their food plant during the growing season. Given the chance, *C. distinctissima* proved to self-select from foods other than *A. alliariae* or *P. albus*, so the expectation that a facultative monophagous species should show less complementary feeding (Pennings et al. 1993) was not corroborated.

Why then were single diets so much less beneficial than the mixed diets? Whereas it is clear that the answer lies somewhere in the interplay between nutrients and secondary compounds contained in the food items, the nutrient and toxin hypotheses did not prove very useful for resolving this question. This is mainly due to the fact that nutritional and toxic effects are not logically distinct categories and therefore the proposed diagnostic tests of the hypotheses are bound to fail. The supposed tests rely on the assumption that total food consumption of a single unbalanced diet cannot be limited to the level of the consumption of a balanced diet, which is clearly not true (Simpson and Simpson 1990; Simpson et al. 1995). Furthermore, the definition of a toxin is unclear, since, in the absence of short-term toxic effects, the detection of toxins relies exclusively on the a priori definition that they cause consumption reductions; however, consumption of an excess of macro- or micro-nutrients can be as toxic as any toxin (Berenbaum 1995). We cannot imagine an easy way to examine the combined effects of nutrients and toxins without precise measurements and extensive experimental manipulation of both. However, a bottom-up integrative approach, based on the measurement of nutritional parameters (the geometrical feeding framework: Raubenheimer and Simpson 1993; Simpson and Raubenheimer 1993b, 1995) provides the ideal basis for that. In our study, the interpretation of utilisation plots (ECI, AD and ECD) allowed us to identify where, in the sequence of events from ingestion to growth (consumption, digestion or postdigestion), single diets differed in their effects on growth, relative to mixed diets.

The elements in this control system which were actually influenced by single diets varied considerably, both within and between the insect species. This means, for instance, that there was no universally toxic or even deterrent food plant among our investigated species. For example, whatever the substance(s) within *L. galeobdolon* which caused *M. alpina* nymphs to die, these had none or at most postdigestive effects on growth in *C. dominula* and preingestive (consumption) effects on *C. distinctissima*. The only positive exception seems to be *A. alliariae* which caused no postdigestive problems in any insect species and was even able to assure good growth at reduced consumption rates in *C. dominula* and *C. distinctissima*. For *M. alpina*, feeding on *S. fuchsii* had exclusively preingestive growth effects, whereas feeding on *U. dioica* had exclusively postdigestive consequences. In *C. dominula*, we found in one case (*F. sylvatica*) a combined preingestive and postdigestive effect on growth, whereas all other single diets, except *A. alliariae*, influenced growth postdigestively. Finally, in *C. dis-*

*tinctissima*, we found one case each of preingestive and postdigestive influence on growth (*L. galeobdolon* and *P. albus*, respectively), as well as one case of the combined action of both (*U. dioica*).

However, one "rule" seems to emerge from the close match of ECI and ECD analyses: once a food was ingested, further effects on growth occurred postdigestively rather than via differential digestibility (egestion). As outlined in Simpson and Raubenheimer (1993a), this is to be expected given that the haemolymph plays a central role in providing the insect with constantly updated information about its nutritional status. Postdigestive processes, however, impose metabolic constraints with the potential of high energetic costs, since all metabolic processing is energy dependent. In our experiment this is shown by all instances where the ECD of single diets lies below the level for the mixed diet.

Therefore one would expect a considerable selection pressure to improve metabolic efficiency, especially in species with low larval mobility which are most likely to get stuck on a less suitable host plant. Efficiency improvement, however, can only be driven to a limit, since detoxification, or dealing with unneeded nutrients, cannot be done without a certain minimal amount of energy. So mixing should be favoured even in a facultative monophagous species (e.g. *C. distinctissima*), if it provides the animal with a suitable blend of nutrients at a lower cost than dealing with nutritional imbalances or toxins in a single diet.

An additional trade-off exists between the risks of foraging for a varied diet (increased predation pressure) and the costs of metabolising a single, less suitable diet. This might be one reason why so many grasshopper species are polyphagous, because they combine high mobility, which facilitates foraging for mixed diets, with an effective predation escape mechanism thereby minimising metabolic as well as foraging costs. Our less mobile lepidopteran species may be able to exploit diet mixing because it evolved an effective predation escape by using a host-plant-derived chemical defence. Only very immobile species may find themselves stuck on less suitable host plants and one would therefore expect the pressure on metabolic efficiency to be greatest in these species, a precondition which should ultimately lead into food specialisation.

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