Intraspecific variation in *Capsella bursa-pastoris* in plant quality traits for insect herbivores

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Summary

The arable plant *Capsella bursa-pastoris* is phenotypically variable in many life history traits, particularly time from germination to flowering. The hypothesis was investigated that, associated with this variation, there are differences in traits that influence plant quality for insect herbivores. Significant within-species variation was found in plant growth, leaf surface characteristics and tissue nutrient composition among 21 accessions of *Capsella*. Short flowering time plants exhibited slow vegetative growth, relatively large shoot nitrogen content, high leaf hair densities and differences in phloem composition, compared with long flowering time plants. Insect herbivore response to distinctive trait combinations was assessed on a subset of seven accessions using the phloem-feeding aphids *Aphis fabae* and *Myzus persicae*. Variation in aphid performance was small but significant, with 15–25% fewer nymphs produced on plant variants that exhibited greater tissue water content and low tissue C:N ratio (*A. fabae*) or on variants with less phloem nitrogen (*M. persicae*). The differential responses exhibited by the two aphid species to the test accessions confirmed that quantifying intraspecific plant variation is a necessary first step in understanding plant functional diversity and its impact on consumers in arable systems.

Keywords: aphid, arable, *Aphis fabae,* herbivore, *Myzus persicae,* functional trait, weed.

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Introduction

Weed species account for a large part of the plant diversity in arable communities (Storkey, 2006) and a diverse array of arable weeds might be necessary to sustain an abundant and diverse array of invertebrate consumers (Stinson & Brown, 1983; Hawes *et al*., 2003). In addition, intraspecific phenotypic variation can be a significant part of the overall variation in arable plant traits influencing resource allocation (Hawes *et al*., 2005). Given the declining abundance of arable weeds associated with intensification of agriculture (Marshall *et al*., 2003; Wilson & King, 2003), this variation could form an important component of resource heterogeneity for herbivores. To understand the functional relevance of this variation to consumers, fine-scale measurement of phenotype at the level of individual plants or groups of individuals from a range of arable sites is required (Diaz & Cabido, 2001; Pachepsky *et al*., 2001; Hawes *et al*., 2005), involving *ex situ* characterisation of plant quality traits under controlled conditions.

Plant quality for consumers depends on chemical and physical traits that affect attraction to, and subsequent performance of, herbivores. Traits that are likely to influence herbivores include plant nutritional composition and the presence of defensive structures and allelochemicals that modify the palatability of plant material or act as feeding deterrents or toxins (e.g. Schoonhoven *et al*., 2005). In highly disturbed arable systems, selection on flowering time, competitive strength and herbicide sensitivity could influence the presence and variability of plant quality traits. An ability to quantify the variation in these traits and then select characterised plant 'types' for experimentation is

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crucial, if we are to understand the functional (i.e. mechanistic) basis for herbivore performance in response to plant phenotypic diversity.

Morphological and physiological variability is known both anecdotally and in the literature for only a few weed species found in arable habitats, including *Stellaria media* (L.) Vill. (Briggs *et al.*, 1991) and *Polygonum aviculare* L. (Meerts & Garnier, 1996). For most arable weeds, the lack of detailed phenotypic information limits assessment of its functional relevance to the plant and invertebrate community. Recently, *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) has been proposed as a model weed for investigating the importance of intraspecific variation to the arable food web (Hawes *et al.*, 2005), because it is common, displays broad phenotypic variation comparable with that of other arable weeds (Aksoy *et al.*, 1999; Hawes *et al.*, 2005; Iannetta *et al.*, 2007) and supports a large number of invertebrate species (Marshall *et al.*, 2003). Detailed characterisation of *Capsella* accessions collected from arable sites throughout the UK has revealed extensive variation in a number of life history traits influencing growth and reproduction. These are associated with differences in flowering time (Fig. 1A) and can be linked to genetic variation (Iannetta *et al.*, 2007). Accessions displaying different phenotypes can co-exist at a given site raising the possibility that *Capsella* offers a heterogeneous resource for associated herbivores in arable systems.

The primary aim of this study was to establish whether the phenotypic variation already reported for *Capsella* extends to plant quality traits for herbivores, by studying in greater detail a subset of accessions representing the range of variation identified previously. The traits that play an important role in the plant–herbivore interaction can be postulated to include the availability of material (plant growth), the provision of *refugia* (plant structure), presence of physical defences (leaf hairs and other structural components), chemical deterrents (glucosinolates predominate in the Cruciferae: Schoonhoven *et al.*, 2005) and nutritional composition of the plant tissue under attack. We conducted an initial study to consider whether trait variation has functional relevance to insect herbivores by testing the responses of two species of phloem feeders, the generalist aphids *Myzus persicae* Sulz. and *Aphis fabae* Scop., to accessions presenting particular combinations of traits.

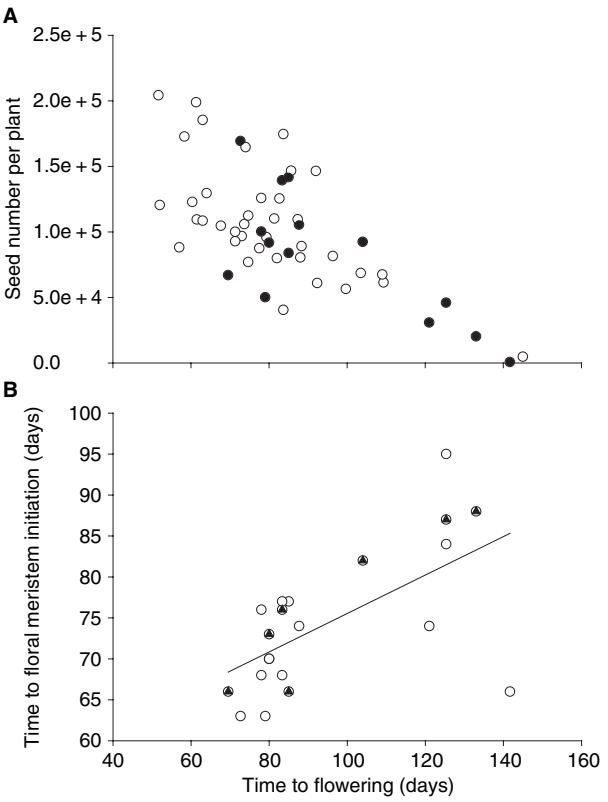

Fig. 1 Flowering time in *Capsella* (A) flowering time and seed production in 53 accessions (unpublished data from Iannetta *et al.*, 2007). Filled circles denote the 14 parental lines of the 21 accessions subjected to further study of physiological and nutritional traits. (B) Time to floral meristem initiation in 21 accessions in relation to flowering time of their parental lines (unpublished data from Iannetta *et al.*, 2007). The correlation coefficient is 0.613; note that two accessions plot to the same point on the graph. Filled triangles denote the seven accessions tested for their impact on aphid performance.

Materials and methods

Plant and insect material

Accessions of *C. bursa-pastoris* were derived from plants emerging from the seedbank sampled in fields throughout the UK used for large-scale experiments on arable biodiversity and food webs (Firbank *et al.*, 2003; Squire *et al.*, 2003). The initial plants were grown to maturity to obtain seed, which was then used in experiments to assess physiological and genetic differences between accessions (Iannetta *et al.*, 2007). Unpublished data on plant traits for the 53 accessions studied by Iannetta *et al.* (2007) were analysed to select 21 accessions representing a wide range in growth and fecundity. Seeds from these accessions were treated with gibberellic acid [GA_3: 0.02% (wt/*V*)] overnight, sown in standard compost (peat–sand–perlite mix containing N:P:K 17:10:15 and Intercept^{TM} systemic insecticide) to minimise seed dormancy and germinated under glass (18 h light, 20°C). At 3 weeks, seedlings were transferred to rehydrated compost pockets (Jiffy^{TM}; Jiffy Products International, As, Norway) and after a further 2 weeks established seedlings were transferred to 10 L pots of standard compost for measurement of plant characteristics. For aphid performance experiments, the compost

was insecticide-free and seedlings were transferred to 2 L pots. Experiments were performed under glass (18:6 h light:dark, 20°C) and plants were irrigated at a rate of 0.025 L water L^{-1} compost day^{-1}; after 8 weeks of growth, plants were fertilised with Sangral Soluble Fertiliser at a proportion of 0.015 L liquid feed L^{-1} compost $week^{-1}$ prepared according to manufacturer's instructions (William Sinclair Horticulture, Lincoln, UK).

Myzus persicae and *A. fabae* are generalist consumers of a variety of crops and weeds including *C. bursa-pastoris* (Aksoy *et al.*, 1998). A clonal culture of *M. persicae* (AJK 04/01) was derived from a single parthenogenetic female collected in July 2004 from an oil seed rape field at SCRI (grid reference NO 337 298). A culture of *A. fabae* (AED 02/32) was collected from *Impatiens glandulifera* L. on the University of York campus (grid reference SE 625 505). Routine cultures of aphids were maintained at 20°C (18 h light) on caged plants or excised leaves of 10 week old plants of *C. bursa-pastoris* belonging to a non-experimental plant accession.

Measurement of physical and nutritional plant characteristics

The 21 accessions of *C. bursa-pastoris* were grown concurrently under glass using a randomised design. For each accession, five replicate plants were sampled immediately prior to flower stem extension, when the flower bud was visible at the centre of the rosette in the majority ($> 50\%$) of replicates. There were three aspects to the measurement of plant characteristics.

Leaf hair density

Abaxial and adaxial leaf hair densities were assessed for a single mature expanded leaf on each plant according to the method of Weyers and Johansen (1985). Duplicate leaf discs were removed from the apex, middle and basal part of the lamina and positive impressions of the abaxial and adaxial surfaces were examined under a light microscope (Zeiss Axioplan 2; Carl Zeiss, Welwyn, UK) to assess the number of leaf hairs per unit area.

Phloem sap sampling using the EDTA exudation technique of King and Zeevart (1974)

From each plant, the petiole of an excised leaf was inserted immediately into 0.2 mL of 1 mM ethylenediaminetetraacetic acid (EDTA) solution (pH 7.5). Samples were incubated for 1 h in an insulated chamber equilibrated at 25°C with a dish of saturated KH_2PO_4 to maintain high humidity. Exudate samples were stored at −20°C prior to chemical analysis.

Leaf and dry matter production and C:N content

Leaf number and rosette diameter were recorded prior to harvesting the shoot material at the base of the rosette. Shoot material was weighed, frozen in liquid nitrogen and freeze-dried for dry mass determination and chemical analysis. Leaf number and dry mass were each divided by the time taken to reach flower bud initiation stage (in days) to give mean rates of leaf production (number day^{-1}) and dry matter accumulation (mg day^{-1}).

Chemical analyses

The sucrose content of phloem exudates was quantified using the method of Dahlqvist (1984). The sucrose present in a 10 μL aliquot of exudate was hydrolysed to completion with 166.7 nanokatal mL^{-1} invertase (cat. no. I-4504; Sigma-Aldrich, Gillingham, Dorset, UK) in 0.05 M Na acetate buffer, pH 4.5 at 37°C for 30 min, and the glucose produced was quantified using the Sigma Diagnostics glucose assay kit (GAGO-20) with glucose standards, following manufacturer's instructions but with *o*-dianisidine concentration altered to 62.5 μg mL^{-1}.

Amino acids in exudates were separated by reverse-phase high-performance liquid chromatography (HPLC) following derivatisation with *o*-phthaldialdehyde (Jones *et al.*, 1981) using a Hewlett-Packard HP1100 series autosampling LC system with ZORBAX™ Eclipse XDB-C8 column and fluorescence detection. Amino acids were quantified by comparison with the AA-S-18 (Sigma-Aldrich) reference amino acid mixture supplemented with asparagine, glutamine and tryptophan. All protein amino acids except proline and cysteine could be detected with this method, with a detection limit of approximately 0.5 pmol, including the nine essential amino acids that cannot be synthesised *de novo* by animals: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Morris, 1991).

Freeze-dried shoot material was ball-milled to a fine powder and the nitrogen and carbon contents of 1 mg samples were determined by continuous-flow Dumas combustion using a Europa Scientific (Crewe, UK) ANCA-SL sample converter and mass spectrometric detection (of N_2 and CO_2) using a Europa Scientific 20-20 mass spectrometer, as described by Scrimgeour and Robinson (2003). The percentage of carbon and nitrogen in the sample was calculated by comparison with known standards.

Aphid performance

The performance of individual aphids was quantified under glasshouse conditions (described above) in

response to a further sub-set of the 21 characterised plant accessions. Seven accessions were chosen that represented a range in flowering time and other traits that were likely to influence plant quality for herbivores: 182/399, 177/475, 474/558, 930/161, 937/71, 798/188 and 799/452. These accessions were significantly different in resource quantity, nutritional quality and plant defences (see *Results*). The performance of newborn nymphs on the abaxial surface of an expanded rosette leaf was assessed on 10 replicate plants (initiated at seven- to 13-leaf stage) following the procedure of Karley *et al.* (2002).

Statistical analyses

Variation in plant traits was explored by principal components analysis (PCA) using a correlation matrix to standardise the variables (Randerath, 1996), and with conversion of phloem amino acid data to relative amounts of each amino acid (mol.%). Parametric statistical tests were applied to data sets confirmed to be normally distributed (Ryan-Joiner one-sample test) with homogeneous variance (Bartlett's test), which occasionally required transformation of the data set, as indicated in the text. Multivariate analysis of variance (MANOVA) was applied to phloem amino acid data (natural log-transformed nmol amino acid exuded). Regression and correlation analyses were applied to test the extent to which variation in aphid performance could be explained by plant traits. When the assumptions of normality and homogeneous variance could not be met, the non-parametric analyses used were Kruskal–Wallis and Spearman rank correlation tests.

Results

Development and resource allocation in C. bursa-pastoris *from arable sites*

The accessions of *C. bursa-pastoris* largely exhibited determinate growth. An initial vegetative growth phase then slowed to an extent that varied among accessions while the plants flowered and fruited over several weeks, after which the plants senesced and died. A comparison of life history traits in all 53 accessions showed a number of differences among particular accessions, but a strong negative relation between time to flowering and seed production was the most apparent (Fig. 1A). Seed production was not related to reproductive duration (i.e. the period from flowering to plant death) and the main factor driving high fecundity was, therefore, short flowering time coupled with greater allocation to reproductive vs. vegetative growth. For the more detailed analysis necessary in the next stage of investigation, seed was bulked by growing the 53 accessions to maturity (described in Iannetta *et al.*, 2007) and a sub-set of 21 accessions was chosen to represent a range of variants in time to flowering and seed production (Fig. 1A). Rate of plant development in the 21 accessions appeared heritable, indicated by the relation between time to floral meristem initiation (TFMI) in accessions chosen for the present study and time to flower opening (TTFO) of their parents: TFMI = 52.1 + 0.24TTFO, $r^2 = 0.38$, ANOVA $P < 0.005$ (Fig 1B).

Plant resource quality at floral meristem initiation

Plant accessions sampled at an equivalent developmental age (floral meristem initiation) exhibited wide variation in traits relating to resource quality for consumers. Principal component analysis was applied to explore the variation in plant traits in relation to rate of development. The first two principal components (PC1 and PC2) accounted for 77% of the variation in the data set (Fig. 2). A plot of the trait attributes (Fig. 2A) revealed that PC1 was related to plant growth rate and density of

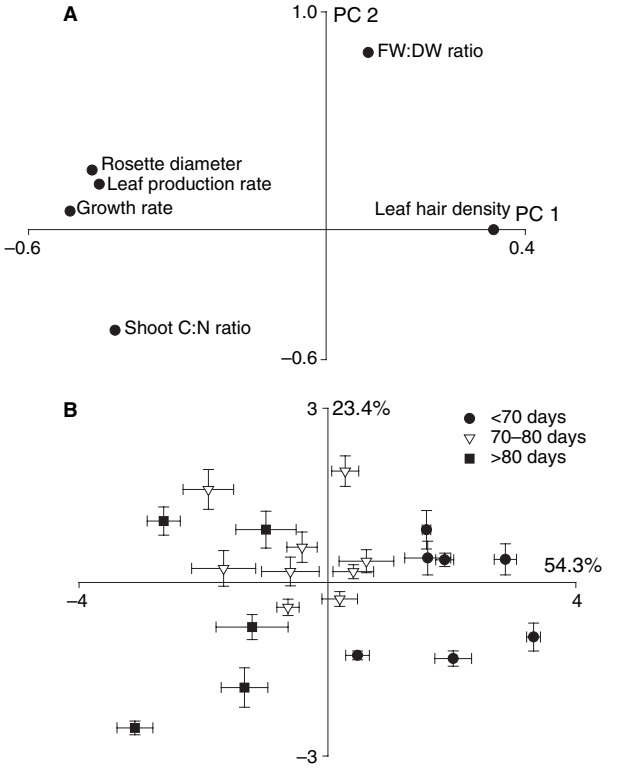

Fig. 2 Principal component analysis of plant quality traits at floral meristem initiation in 21 accessions of *Capsella*. (A) Attribute loadings on the first two components PC1 and PC2 (see methods for explanation of attribute annotations). (B) The mean (±SE, n = 3–5) sample scores for each accession plotted onto PC1 and PC2, which explain 54.3% and 23.4% of the variation in the data set, respectively, are grouped by time to floral meristem initiation.

leaf hairs, while PC2 was related to plant fresh:dry matter ratio and relative nitrogen content. These two axes tended to separate short flowering time types (small plants with relatively high leaf hair density and shoot nitrogen content) from longer flowering time types (large, fast-growing plants with relatively low leaf hair density and shoot nitrogen content), as indicated when plants were assigned to arbitrary time to floral meristem initiation groupings (<70, 70–80 and >80 days) on the first two principal components (Fig. 2B). Note that abaxial leaf hair density (not shown) was significantly positively correlated with adaxial leaf hair density (Spearman rank correlation coefficient = 0.529, $P < 0.001$).

Phloem exudates sampled from plants at the floral meristem initiation stage were analysed for amino acid composition. There was significant variation among the 21 accessions in their amino acid composition (MANOVA $P < 0.001$). Visual exploration of the amino acid data by PCA revealed that PC1 (accounting for 32.9% of the variation in the data set) separated glutamine and glycine from all other amino acids, particularly serine, threonine and some of the other essential amino acids (see methods for classification of essential and non-essential amino acids). Arginine and a number of essential amino acids (e.g. methionine, valine) were separated along PC2 (20% of the variation; Fig. 3A). A plot of the score values indicated that these two axes separated short flowering time accessions, whose phloem exudates were influenced by the non-essential amino acids glutamine and glycine, from long flowering time accessions whose exudates contained relatively larger proportions of most essential amino acids and the non-essentials arginine, serine and tyrosine (Fig. 3B).

Analysis of individual plant traits revealed significant variation between plant accessions and, for most traits, between flowering-time groups (Table 1). Regression analysis was used to explore the relation between phloem composition and the other plant traits shown in Fig. 2. Variation in the scores on PC2 correlated significantly with shoot C:N ratio (PC2 scores = $2.92 \times$ shoot C:N $- 17.2$, $r^2 = 0.46$; ANOVA $P < 0.005$; Fig. 4A), showing a clear trend that also related to flowering time. Phloem sucrose:amino acid (C:N) ratio and scores on PC1 of the phloem analysis showed no significant relation with plant growth, leaf hair density or shoot nutrient content.

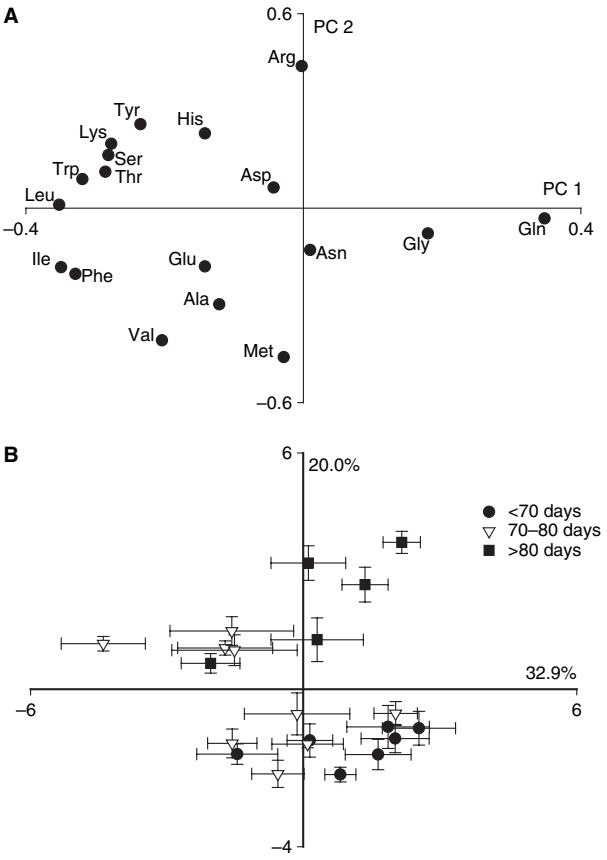

Fig. 3 Principal component analysis of amino acid mol% data in leaf phloem exudates sampled at floral meristem initiation in 21 accessions of *Capsella*. (A) Attribute loadings on the first two components PC1 and PC2. (B) The mean (±SE, $n = 5$) sample scores for each accession plotted onto PC1 and PC2, which explain 32.9% and 20.0% of the variation in the data set, respectively, are grouped by time to floral meristem initiation. Amino acid concentrations were converted to mol.% prior to analysis. Standard abbreviations are: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Glu, glutamate; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

Insect performance

All aphids (except one *A. fabae* individual) settled, fed and produced offspring on leaves, indicating that the seven accessions chosen for testing insect performance (Table 2) were acceptable to both *A. fabae* clone AED 02/32 and *M. persicae* clone AJK 04/01. Plant type had no significant effect on the time taken for the offspring to reach adulthood (median value of 9 days for both clones) or to initiate reproduction (median value of 10 days for both clones; statistical analyses not shown). For the two aphid clones, plant type had no impact on nymph production in the first few (up to 4 or 6) days of reproductive life, but there were differences between the species in their subsequent performance. The estimated intrinsic rate of population increase (R_m: data not shown) was significantly altered by plant type in *M. persicae* (Kruskal–Wallis $P < 0.05$), but not in *A. fabae* (ANOVA $P > 0.1$). A plot of cumulative nymph production by the two clones indicated that reproductive output of *M. persicae* clone AJK04/01 was generally

Table 1 Analysis of trait variation for 21 accessions of *Capsella* and relation to plant development rate

	Shoot growth rate (mg dry mass day^{-1})	Fresh:dry mass ratio	Leaf production (number day^{-1})	Rosette diameter (mm)	Shoot C:N	Adaxial leaf hair density	Phloem C:N*	Phloem PC1 scores	Phloem PC2 scores
ANOVA (accessions)	$H_{20} = 93.11$† $P < 0.001$	$F_{20,104} = 24.94$ $P < 0.001$	$F_{20,104} = 13.51$ $P < 0.001$	$F_{20,104} = 26.49$ $P < 0.001$	$F_{20,104} = 21.25$ $P < 0.001$	$H_{20} = 56.83$†‡ $P < 0.001$	$H_{20} = 35.24$† $P < 0.05$	$F_{20,104} = 4.57$ $P < 0.001$	$H_{20} = 84.95$† $P < 0.001$
ANOVA (time to floral meristem initiation groups)	$F_{2,20} = 32.11$ $P < 0.001$	$F_{2,20} = 2.49$ $P > 0.1$	$F_{2,20} = 19.48$ $P < 0.001$	$F_{2,20} = 9.17$ $P < 0.005$	$F_{2,20} = 7.10$ $P < 0.005$	$F_{2,20} = 4.15$‡ $P < 0.05$	$F_{2,20} = 0.84$ $P > 0.1$	$F_{2,20} = 3.67$ $P < 0.05$	$H_2 = 10.91$† $P < 0.005$

See Appendix 1 and Fig. 2 for original data.
*Phloem C:N ratio is equivalent to the molar ratio of sucrose:amino acid.
†Kruskal–Wallis analysis.
‡Analysis performed on $\log_e$-transformed data.

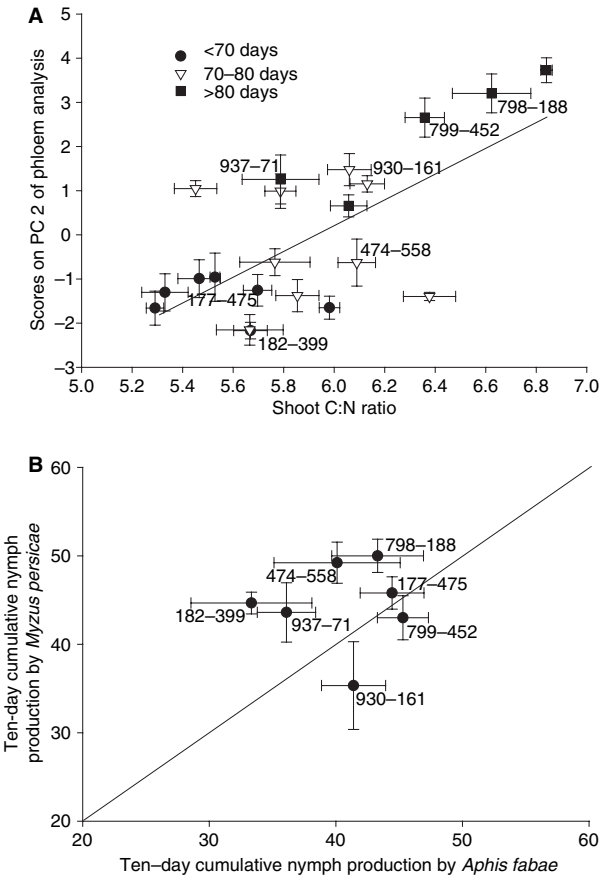

Fig. 4 (A) Phloem composition and shoot C:N ratio for 21 accessions of *Capsella* assessed at floral meristem initiation. Mean values (±SE, $n = 5$) are grouped by time to floral meristem initiation. The solid line indicates the regression: PC2 scores $= 2.92 \times$ shoot C:N $- 17.2$, $r^2 = 0.46$. The accessions tested for aphid performance are annotated; note that 182-399 refers to the underlying filled circle symbol. (B) Cumulative nymph production by *Aphis fabae* and *Myzus persicae* aphids developing from offspring raised on seven different accessions of *Capsella*. Points with equivalent values for the two species fall on the solid line. Values are mean (±SE) of $n = 7$–10.

similar to, or higher than, that of *A. fabae* clone AED02/32 in response to the seven *C. bursa-pastoris* accessions (Fig. 4B). Cumulative offspring output was reduced for the *A. fabae* clone on accessions 182/399 and 937/71 from day 5 onwards, and this variation was significant for day 6 (Kruskal–Wallis $P < 0.05$) of reproduction. For *M. persicae* clone AJK 04/01, a reduction in nymph output was observed on accession 930/161, and this variation was significant from day 7 (Kruskal–Wallis $P < 0.05$) to the end of the observation period on day 10 (Kruskal–Wallis $P < 0.05$). On average, the least successful aphid–plant type combinations produced 15–25% fewer nymphs over the 10 day reproductive period compared with the other plant types.

Plant traits that might underlie between-accession variation in aphid performance were identified by

regression of mean values for cumulative nymph output (Fig. 4B) on mean values for plant traits (Table 2) and tested by correlation analysis. Although positive and negative trends were identified, correlations were non-significant at $P = 0.1$, except for the associations between *M. persicae* nymph output and phloem C:N (nymph output = $53.3 - 3.96 \times$ phloem C:N, $R^2 = 47.1\%$; $C = -0.687$, $P = 0.088$) and phloem composition (nymph output = $44.5 + 1.56 \times$ phloem PC1 scores, $R^2 = 45.0\%$; $C = 0.671$, $P = 0.099$).

Discussion

Plant quality traits varied significantly among a selection of functionally distinct *C. bursa-pastoris* accessions collected from arable sites across the UK. Intra-specific variation in *C. bursa-pastoris* has been investigated typically on the basis of leaf morphology (Aksoy *et al.*, 1999), but recent research indicates that leaf shape is linked only weakly to traits such as growth and fecundity and that plant development rate, particularly flowering time, offers an alternative functionally relevant classification (Iannetta *et al.*, 2007). A notable finding of the present study was the strong association of plant development both with productivity traits such as seed production (Fig. 1A) and with quality traits, particularly tissue and phloem nutrient content (Fig. 4A). While the genetic basis of flowering time adaptation has yet to be determined definitively for *C. bursa-pastoris*, genes involved in the regulation of the circadian clock *CCA1* and *TOC1* have been implicated (Slotte *et al.*, 2007); in a similar species, *Arabidopsis thaliana* (L.) Heynh., selection upon the key flowering time genes, *FRI* and *FLC* may also contribute to determinants of fecundity (Korves *et al.*, 2007).

Notable relationships existed in the measured traits. Short-flowering accessions exhibited lower rates of primary production (i.e. resource for consumers), but higher seed output and shoot nitrogen content than the long flowering time lines. Short flowering time therefore appears favourable and might explain the overall dominance of these types in UK arable systems (Iannetta *et al.*, 2007). However, small plants with high nitrogen requirements might be expected to compete weakly with crop plants and to provide a rich nitrogen source for herbivores in spring and summer. This could have ecological significance for regional variation in the abundance and performance of *C. bursa-pastoris* and its consumers, as arable systems in northern UK tend to support higher proportions of long flowering time types (Wishart *et al.*, 2007).

These properties of the whole plant were associated with traits that might directly mediate interactions with specific herbivores. For example, greater shoot nitrogen

Table 2 Plant and phloem traits of seven accessions of *Capsella bursa-pastoris* selected for testing aphid performance

Accession	Days to floral meristem initiation	Shoot growth rate (mg dry mass day^{-1})	Fresh:dry mass ratio	Leaf production (number day^{-1})	Rosette diameter (mm)	Shoot C:N	Adaxial leaf hair density*	Phloem C:N	Phloem arginine (mol.%)	Phloem glutamine (mol.%)
182/399	66	7 ± 1	8.67 ± 0.16	0.17 ± 0.01	234 ± 17	5.67 ± 0.07	14.0 ± 1.4	1.95 ± 0.42	1.4 ± 0.5	31.1 ± 0.7
177/475	66	17 ± 2	7.95 ± 0.14	0.28 ± 0.02	332 ± 12	5.70 ± 0.06	10.7 ± 2.3	2.64 ± 0.21	2.2 ± 0.3	40.7 ± 4.6
474/558	73	39 ± 5	8.97 ± 0.06	0.33 ± 0.02	512 ± 27	6.09 ± 0.07	6.3 ± 0.8	2.00 ± 0.69	3.4 ± 0.6	36.5 ± 6.3
930/161	76	51 ± 3	8.69 ± 0.10	0.51 ± 0.05	478 ± 8	6.13 ± 0.07	2.8 ± 0.9	3.92 ± 1.08	6.5 ± 0.4	23.4 ± 1.8
937/71	82	92 ± 9	9.43 ± 0.31	0.66 ± 0.04	496 ± 18	5.79 ± 0.15	6.8 ± 1.2	1.34 ± 0.23	5.8 ± 1.0	38.0 ± 2.8
798/188	87	77 ± 6	7.72 ± 0.27	0.51 ± 0.03	450 ± 31	6.62 ± 0.16	4.8 ± 0.8	1.96 ± 0.51	12.4 ± 1.3	30.4 ± 2.3
799/452	88	79 ± 12	8.40 ± 0.15	0.53 ± 0.05	528 ± 35	6.36 ± 0.08	5.0 ± 0.8	1.76 ± 0.21	9.5 ± 1.3	37.7 ± 2.5
ANOVA		$H_6 = 29.59$†	$F_{6,34} = 9.77$	$F_{6,34} = 22.33$	$F_{6,34} = 22.21$	$F_{6,34} = 12.66$	$H_6 = 18.07$†	$H_6 = 10.01$†	$H_6 = 28.86$†	$H_6 = 13.77$†
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P > 0.1$	$P < 0.001$	$P < 0.05$

Values are mean (±SE) of $n = 5$, except for leaf hair density measurements, which are mean values of $n = 3$–5 values averaged across three points along the leaf blade.
*Number of leaf hairs per 17 mm^{-2}.
†Kruskal–Wallis analysis.

concentration in the short-flowering accessions might be linked to greater investment in leaf hairs as a physical defence (e.g. Hanisch, 1981). Interestingly, decreased shoot relative nitrogen concentration also correlated with an increase in the scores on PC2 in parallel with lengthening flowering time (Fig. 4A). The PC scores incorporated both increases and decreases in nitrogen-rich amino acids like arginine, glutamine and asparagine (Fig. 3) and tissue C:N might relate to, or reflect, the source and availability of particular amino acids for phloem nitrogen transport. Although leaf nitrogen concentration does not quantitatively reflect phloem nitrogen content (this study; Douglas, 1998), the possibility that tissue C:N might be diagnostic of phloem composition and nutritional quality for phloem feeders warrants further investigation. The key phloem compositional differences between fast- and slow-developing plants (i.e. essential amino acids and the non-essentials arginine and glutamine: Fig. 3) can have significant effects on phloem-feeder performance (discussed below) and are specific both to plant development stage (Karley *et al*., 2002) and genotype (Weibull, 1988). In this study, a single developmental stage was sampled (i.e. at floral meristem initiation), and analysis at other growth stages would confirm whether the differences between accessions are maintained throughout their development. Glucosinolates were not detected in analyses of *C. bursa-pastoris* shoot tissue (analysis not shown), consistent with previous analyses of leaf surface glucosinolates in this species (Griffiths *et al*., 2001).

We not only found that two aphid species, *A. fabae* and *M. persicae*, showed differential responses to individual accessions of *C. bursa-pastoris*, but also that, despite large variation in these accessions, the differences in aphid performance were small (Fig. 4B). For each aphid species, small reductions in aphid fecundity were observed on at least one plant line. Cumulative reproductive output of *A. fabae* was lowest on the late-flowering line 937/71, with a relatively high water content (Table 2), and on short-flowering plant line 182/399, with low tissue C:N and high leaf hair densities (Table 2), which can impair aphid feeding and success (Webster *et al*., 1994). By contrast, the fecundity of *M. persicae* was impaired on the intermediate-flowering line 930/161 with low phloem nitrogen and particularly low phloem glutamine levels (Table 2); these traits have been associated with poor aphid performance in other studies (Weibull, 1988; Karley *et al*., 2002). While some of the traits were accession-specific (see Table 2) and could be responsible for poor aphid performance, other trait combinations were not (e.g. the short-flowering line 177/475 also exhibits low tissue C:N and high leaf hair density but does not reduce the performance of *A. fabae*: Table 2), and more detailed performance measures in response to experimental manipulation of individual traits are required to identify the traits or trait combinations that determine the aphid response. Although not investigated here, within-species (interclonal) variation in aphids can differentially affect their responses to plant quality traits (e.g. Karley *et al*., 2002; Wilkinson & Douglas, 2003), altering the outcome of competition between aphid species (Hazell *et al*., 2006).

This study demonstrated that variation in plant development rate could indicate plant types differing systematically in physiological characteristics that alter the quality of plants as food for herbivores. However, we could identify no simple relationship between plant flowering time and quality of resource for phloem feeders in our initial study of two aphid species. Differential aphid performance on the seven tested accessions of *C. bursa-pastoris* can be interpreted as the insect response to combinations of plant traits that conceal clear associations with individual plant features. Plant water status, the quantity and composition of tissue nitrogen, and leaf hair density were identified as the focus for future studies that should include a wider range of insect herbivores to confirm the functional relevance of plant trait variation. More generally, decreased plant water status has an adverse effect on sap-feeder and gall-former performance, despite accompanying increases in plant nitrogen status and phloem amino acid concentration, but has little effect on chewing insects (Girousse *et al*., 1996; Huberty & Denno, 2004). Relative nitrogen content and tissue nitrogen quality are important determinants of the performance of aphids (Douglas, 1993, 1998; Simpson *et al*., 1995) and other insect herbivores (Mattson, 1980; Schoonhoven *et al*., 2005). Extending our research beyond phloem-feeders would identify whether the traits defined here impose a selection pressure on seed feeders, leaf-chewers and other herbivore types.

This study builds on previous research to establish *C. bursa-pastoris* as a representative arable weed for studying the functional importance of plant traits in agroecology (Hawes *et al*., 2005; Iannetta *et al*., 2007; Wishart *et al*., 2007), with the aim of extending our studies to other herbivore groups as well as quantifying functional diversity among insect consumers. In particular, we wish to determine whether the breadth of variation detected in arable accessions of *C. bursa-pastoris* (this study; Iannetta *et al*., 2007; Wishart *et al*., 2007) is a characteristic of other arable weeds. A full description of the partitioning of functional variation within- and between-species has not, to our knowledge, been attempted for the arable habitat. However, in a recent eco-physiological analysis of trait variation between single genotypes of arable weed species, *C. bursa-pastoris* occupied a position on the trait matrix close to other common annual weeds, such as

S. media and *Senecio vulgaris* L. (Storkey, 2006). We therefore anticipate considerable trait overlap between ruderal species (Hawes *et al*., 2005), and extension of our trait-based approach to other arable species would test this hypothesis. Ultimately, we envisage a framework that involves identifying particular plant traits or associations of traits that have predictable consequences for different herbivores, permitting functional analysis at the sub-species and individual level.

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Appendix

Plant traits relevant to above-ground insect herbivory for 21 accessions of *Capsella*

Accession	Days to floral meristem initiation	Shoot growth rate (mg dry mass day^{-1})	Fresh:dry mass ratio	Leaf production (number day^{-1})	Rosette diameter (mm)	Shoot C:N	Adaxial leaf hair density	Phloem C:N
156/230	63	18 ± 4	9.22 ± 0.12	0.40 ± 0.03	322 ± 27	5.33 ± 0.09	3.9 ± 0.9	2.8 ± 0.7
280/509	63	31 ± 2	9.64 ± 0.20	0.38 ± 0.04	427 ± 14	5.47 ± 0.08	10.8 ± 1.6	1.3 ± 0.2
792/	66	13 ± 3	9.54 ± 0.25	0.19 ± 0.03	324 ± 21	5.29 ± 0.03	10.3 ± 1.9	2.6 ± 0.6
177/475	66	17 ± 2	7.95 ± 0.14	0.28 ± 0.02	332 ± 12	5.70 ± 0.06	10.7 ± 2.3	2.0 ± 0.4
182/399	66	7 ± 1	8.67 ± 0.16	0.17 ± 0.01	234 ± 17	5.67 ± 0.07	14.0 ± 1.4	2.6 ± 0.2
930/126	68	27 ± 2	8.01 ± 0.05	0.46 ± 0.03	364 ± 4	5.98 ± 0.04	4.0 ± 1.4	3.0 ± 0.5
469/260	68	27 ± 2	9.32 ± 0.09	0.40 ± 0.03	396 ± 13	5.53 ± 0.02	14.3 ± 2.0	2.7 ± 0.4
474/555	70	38 ± 4	9.14 ± 0.08	0.33 ± 0.02	492 ± 26	5.77 ± 0.14	4.6 ± 0.8	1.2 ± 0.3
474/556	70	36 ± 7	9.18 ± 0.08	0.42 ± 0.07	480 ± 30	5.67 ± 0.13	6.8 ± 0.6	1.9 ± 0.2
474/558	73	39 ± 5	8.97 ± 0.06	0.33 ± 0.02	215 ± 27	6.09 ± 0.07	6.3 ± 0.8	2.0 ± 0.7
158/418	74	60 ± 9	8.79 ± 0.18	0.49 ± 0.03	580 ± 30	5.85 ± 0.09	6.0 ± 1.9	3.0 ± 0.6
153/423	74	71 ± 6	9.07 ± 0.19	0.81 ± 0.09	510 ± 13	6.38 ± 0.10	7.8 ± 1.4	3.8 ± 0.9
469/261	76	50 ± 5	10.41 ± 0.21	0.47 ± 0.03	538 ± 19	5.45 ± 0.08	5.8 ± 1.0	2.6 ± 0.5
930/161	76	51 ± 3	8.69 ± 0.10	0.51 ± 0.05	478 ± 8	6.13 ± 0.07	2.8 ± 0.9	3.9 ± 1.1
930/165	77	51 ± 5	9.39 ± 0.20	0.57 ± 0.05	465 ± 16	5.79 ± 0.06	2.1 ± 0.9	1.6 ± 0.4
167/471	77	91 ± 9	10.18 ± 0.24	0.65 ± 0.05	634 ± 25	6.06 ± 0.09	4.2 ± 0.6	2.2 ± 0.6
937/71	82	92 ± 9	9.43 ± 0.31	0.66 ± 0.04	496 ± 18	5.79 ± 0.15	6.8 ± 1.2	1.3 ± 0.2
798/424	84	128 ± 8	9.44 ± 0.15	0.60 ± 0.05	670 ± 19	6.06 ± 0.07	3.1 ± 0.9	2.8 ± 0.3
798/188	87	77 ± 6	7.72 ± 0.27	0.51 ± 0.03	450 ± 31	6.62 ± 0.16	4.8 ± 0.8	2.0 ± 0.5
799/452	88	79 ± 12	8.40 ± 0.15	0.53 ± 0.05	528 ± 35	6.36 ± 0.08	5.0 ± 0.8	1.8 ± 0.2
798/189	95	112 ± 9	6.57 ± 0.13	0.58 ± 0.04	594 ± 9	6.84 ± 0.02	4.4 ± 0.4	1.9 ± 0.5

Values are mean (±SE) of $n = 5$, except for leaf hair density measurements, which are mean values of $n = 3$–5 values.

