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Variation in *Capsella* (shepherd's purse): an example of intraspecific functional diversity

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The extent of functional trait diversity is quantified for 157 different *Capsella bursa-pastoris* (L.) *medic* (shepherd's purse) accessions. These individuals encompass replicate progeny generated from seed of 53 different *Capsella* 'maternal lines' that were isolated at random as they emerged from soil cores (used to estimate baseline seed bank numbers and weed diversity) at 34 different arable sites across the United Kingdom. The replicate progeny were subject to ex situ characterisation for traits determining life history and architecture. Seven leaf-type classes were identified and representative parents of each leaf type were distinguishable using four different simple sequence repeat markers. Life-history traits were only loosely associated with leaf shape, and cluster analysis grouped the accessions into three broad types: small plants that flowered early with intermediate reproductive output; large plants, with intermediate time to flowering and a high reproductive output; late-flowering plants, of intermediate size and low reproductive output. The most common leaf-type variants (83% of accessions) demonstrated a short time to flowering (ca. 70 days), while rarer variants included those that flowered after 140 days, accumulated more nitrogen and produced less seed: possibly representing advantageous and disadvantageous traits (respectively), in modern arable rotations. A wide trait variation was therefore found in *Capsella bursa-pastoris* despite decades of agricultural intensification, the range of time-to-flowering for *C. bursa-pastoris* being as broad the mean flowering times of the commoner annual and winter annual arable species. We propose the use of traits, rather than species, as the accounting unit to quantify functional biodiversity in arable systems.

Introduction

Farmed ecosystems have suffered some of the greatest reductions of wild plant biodiversity in the last 50 years (Marshall et al. 2003), and the potential impact of such diversity losses upon the function of this ecosystem has raised concerns. Consequently, and to address this issue, a fundamental question is borne out of this scenario, namely, '*what is the link between wild plant diversity and ecosystem function?*'.

Production ecosystems, such as arable land and grassland, are habitats with the special characteristic: specifically, that a large part of their primary production is removed as yield. They nevertheless depend on enough primary production being left to support organisms that mediate essential processes such as decomposition, nutrient transformations, soil formation and regulation of herbivore populations. Since agriculture began, crops and other plants, the weeds, have both contributed to this

Abbreviations – EEI model, diagonal with equal volume and equal shape; GA_3, gibberellic acid; SD, standard deviation; SPAD, Single Photon Avalanche Diode; T_{50}, time for 50% of germination.

primary production. During the second half of the 20th century, new varieties and improved agronomy had raised the potential yield of all the major crops by an order of 1% a year (Evans 1993). By the late 20th century in Britain, annual production by well-managed crops was typically 1500 g m^{-2} (Gallagher and Biscoe 1978, Monteith 1977, Werker and Jaggard 1998), while the dry mass of the weeds had been reduced to around 1% of this, or around 15 g m^{-2} (Heard et al. 2003, Squire et al. 2003). The seedbank, the source and buffer of the arable flora, had declined at about 3% a year since 1950 (Hawes et al. 2005), and many less common arable plant species were in serious decline, some to near extinction (Marshall et al. 2003, Preston et al. 2002), as were many invertebrates and birds. It is nevertheless still uncertain whether this dominance of crop production and the relegation of other plants is disrupting properties of the managed system, such as retaining nutrients, harbouring mycorrhizae, supporting pollinators, resisting invasions by other plants and sheltering predators of crop pests.

The plants contribute mass in the form of roots and root exudates and leaf litter. There are around eight relatively common species of crop, dominated by autumn-sown wheat and barley that occupy around 80% of the arable, in-field surface, and around 300 species of weed, in the Chenopodeaceae, Asteraceae, Polygonaceaa, Poaceae, Brassicaceae and many other families, only a handful of which are pernicious to yield. In addition, crops are generally grown in sequence of monocultures, not in mixtures, while the weeds potentially form mixed stands with each other and the current crop. While the importance of botanical diversity to function is accepted in theoretical terms (Loreau 1998, 2000), and has been demonstrated within specific habitats such as grassland (Koricheva et al. 2000, Siemann et al. 1998), it is not understood for the arable habitat. Certainly, a weed flora can be tolerated in and around cereal crops and related breaks such as oilseed rape, because many agronomic experiments in the 1990s show negligible improvements in yield by manipulating the weed biomass around the current 1% (Young et al. 2001). It is also well established that this low wild plant biomass feeds and supports many more types of invertebrates than the crops themselves (Hawes et al. 2003, Norris and Kogan 2000, Stinson and Brown 1983). Experimentally, the problem should be addressed by manipulating plant diversity and observing the effect on important processes.

Effective measurement of ecologically meaningful plant-biological variation demands that the unit or metric of diversity be defined. To date, diversity in the arable flora has been defined through the use of taxonomic units such as species or genus (Lavorel et al. 1997) from which functional properties are sometimes inferred. Even the more sophisticated of such applications (e.g. Hooper et al. 2000) ignore the high levels of variability that exist within species (Hawes et al. 2005). Accordingly, the effect of biodiversity upon system function would be better described through the relative abundance of ecologically significant plant life-history traits than taxonomic units (Diaz and Cabido 2001, Pachepsky et al. 2001). At present, so little is known of variation within arable species that such questions cannot be addressed. There is need therefore to quantify the extent of within- and between-species variation in traits linking plants with environment in these strongly perturbed habitats, where traits such as those driving phenology and the allocation of resource between reproductive and vegetative structures are particularly important (Charnov 1982, Ghersa et al. 1994, Neuhauser et al. 2003). Assessing function through traits would moreover allow the theoretical prediction (Pachepsky et al. 2001) to be tested: that the link between biodiversity and ecosystem functioning can be examined at small experimental scales.

Here, the unit of functional diversity is examined by seeking and characterising forms of a widespread arable plant, *Capsella bursa-pastoris* (L.) *medic*, previously identified as an important ecological 'model' in this habitat because of its widespread occurrence, importance to the food web and synteny with the genetic model *Arabidopsis thaliana* (Hawes et al. 2005). Leaf-shape variants (Aksoy et al. 1999, Clapham et al. 1987, Shull 1909, 1914, 1918, 1923, 1929, Tutin et al. 1993) in this and the related *Capsella rubella* (L.) *medic* have been used for almost 100 years as an indicator of diversity within this species. An association between leaf shape and life-history traits has been implicated but not confirmed (Aksoy et al. 1999, Meikle 1977, Neuffer and Eschner 1995, Svensson 1983).

The first task in this study was to determine whether variants of *Capsella* remain in arable fields in Britain. As a direct link with previous work on *Capsella*, we ask, first '*are the leaf-shape variants distinguished by Shull and others common within the seedbank?*'. Accordingly, *Capsella* accessions obtained from arable seedbanks throughout the United Kingdom were characterised ex situ, according to principles advocated by Gaudet and Keddy (1988). This approach allowed us to address two further questions: second, '*are the variants genetically distinct, for example* via *proven association with genetic markers?*' (cf. Mitchell-Olds 2001); and third, '*is any such leaf-shape variation likely to be functionally relevant in the disturbed habitat, by association with specific life-history traits such as time-to-flowering, and thereby liable to affect element-cycles and food web resources?*'. If the answers to these questions are positive, the way is open to use such variants to examine links experimentally

between functional types and properties of the habitat in situ or in constructed communities.

Materials and methods

Plant material

Capsella seedlings were isolated as they emerged from soil samples taken from 34 fields among the Farm Scale Evaluation sites in 2001 (Champion et al. 2003, Heard et al. 2003). Field sites were not preselected and represented a wide range of geographical locations from the north-east of Scotland to southern England. Sites also differed in their management intensity, the latter indicated by both low and high seedbanks of between 1000 and 17 000 m^{-2} (Squire, unpublished data). Up to four of the individual *Capsella* plants from each field were grown to provide seed from in total 131 accessions. Harvested seed was treated identically and stored to optimise viability (dark/15% relative humidity/4°C). Accessions were allocated to one of three leaf-shape categories (1) simplex, (2) rhomboidea or (3) heteris/tenuis (Fig. 1A) based on Shull's original classification. For the following physiological study, a subset was selected: one accession for each leaf shape (if found) from each site, to provide 53 parents with a minimum of three progeny per accession. At the 33 sites, most contained leaf forms (1) and/or (2) (ca. 14 and 25 sites, respectively). Only two sites produced *Capsella* demonstrating all three leaf forms. Note also that flow-cytometry analysis of the 53 parent lines identified them as tetraploid (*C. bursa-pastoris*); the diploid, *C. rubella* was not found (data not shown).

Growth conditions

Capsella seeds subject to sterile culture were germinated on 1% sterile distilled water agar and stratified for 3 days (4°C) and cultured at 21°C. One-week-old seedlings were transferred to small peat pots (6 weeks) before transferring to large 15-L peat containing pots and were provided with water daily (150 ml), 150 ml of liquid fertiliser [10% (w/v) '20:20:20 Sangral Soluble Fertiliser' (William Sinclair Horticulture Ltd, Lincoln UK)] every 7th day. Plants were provided with 18 h of daylight (>250 W m^{-2}) and a day/night temperature regime of 25/15°C.

Phenotypic measurements

Rosette diameter, leaf number and leaf shape were recorded when flowering primordeum became apparent. Leaf shape can only be reliably attributed using leaves from ninth node (or more) and were therefore used to assign specific leaf class. Date of flowering was recorded as time from germination. Flowering stems were isolated to promote self-fertilisation (CryovacUK Ltd, Sandy, Bedfordshire UK) and flowering stems were recorded and harvested when siliculae showed the first signs of pod dehiscence. Reproductive duration is the time from first flowering to flowering-stem harvest. Chlorophyll fluorescence was measured using a 'Single Photon Avalanche Diode'-containing (SPAD) meter. Three SPAD readings were made on each of three different randomly selected, largest/most-mature leaves every 7 days until the flowering primordeum was apparent.

Three individuals per parental line were randomly selected to assess fecundity. Stem dry weight was recorded after oven-drying (70°C/3 days). Total seed weight was recorded and 500 seeds removed for seed size by digital measurement [Fuji FinePix S1 Pro (3.2 mega pixel) digital camera and Micro Nikkor 55-mm lens]. The parameters recorded in pixels were converted to millimetres (1 mm = 51.63 pixels) and analysed electronically using SCION Image). Seed germination characteristic (viability and time to 50% germination) was assessed using two subsamples, treated overnight in either sterile distilled water or 0.05% (w/v) gibberellic acid (GA_3), before germination in peat and under the growth conditions already defined. Seed viability was the number of seedlings emerging from the plus-GA_3 treatment as a percentage of the number sown. The time for 50% of germination (T_{50}) of viable seeds was estimated from the minus-GA_3 treatment. The frequency of emerging seedlings increased and decreased in a cyclic fashion. Therefore, the number of 'flushes of germination' for each seed sample was also noted. Dormancy was estimated as the number of viable seeds that failed to germinate under minus-GA_3 conditions as a proportion of the estimated total number of viable seeds.

Statistical analysis of phenotypic data

There was an unbalanced experimental design as parental leaf shape did not always match that of their progeny: 12 of the 53 maternal plants contributed offspring of two or more leaf shapes. In addition, the presence of maternal lines introduces dependence between individuals. Consequently, a multistratum analysis of variance assessed differences in functional traits between leaf variants at two levels: between maternal lines and between individuals within a maternal line. Additionally, it should be noted that leaf variant is partially confounded with the site of origin for the maternal lines, especially for rare leaf variants, which were obtained from just one site. A further test of the association between leaf variant and the functional traits was made by the construction of a classification tree

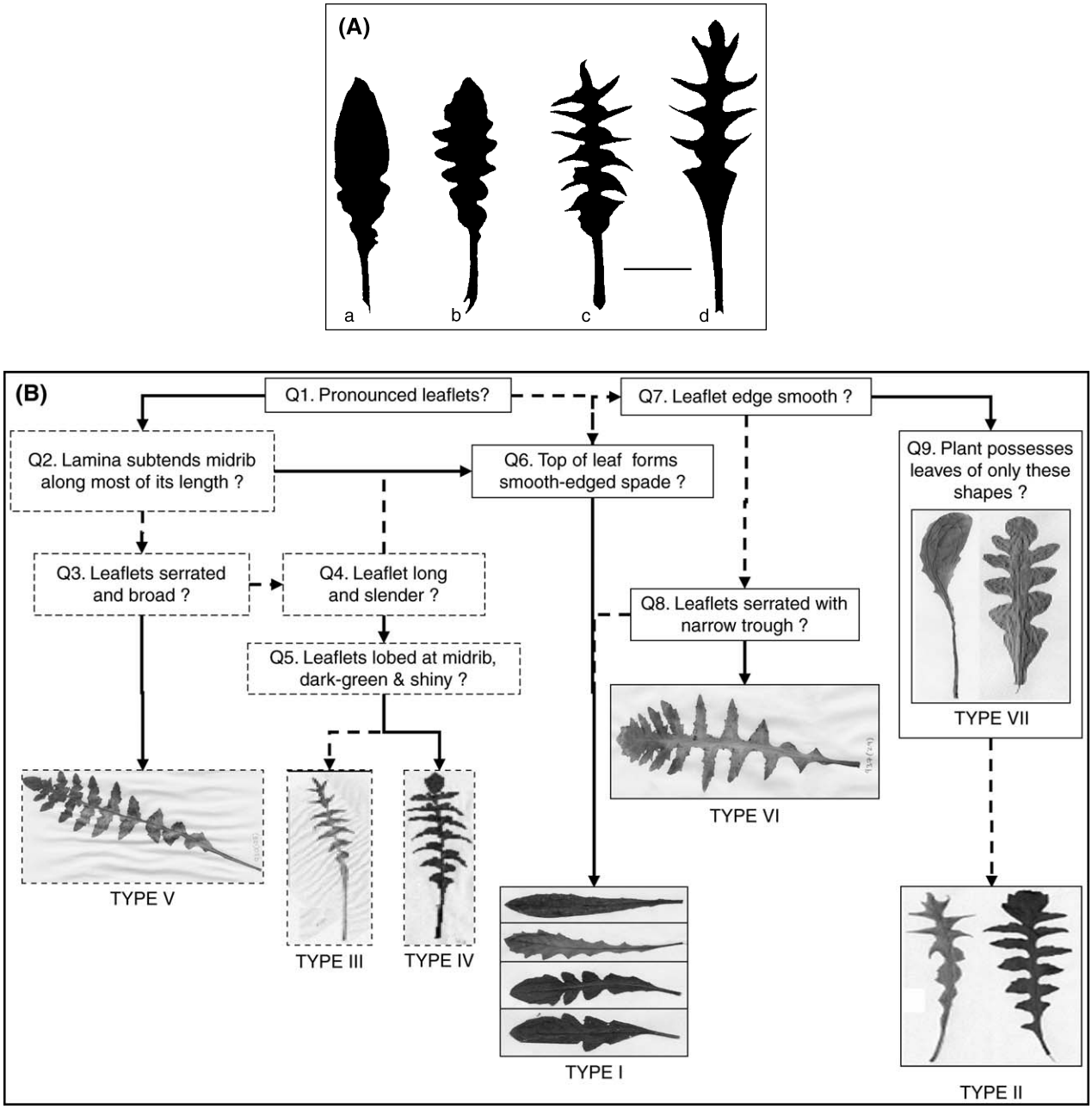

Fig. 1. (A) The four leaf shapes stereotyped by sensu Shull (1909) for F_3 progeny of *Capsella* species grown under controlled-environment conditions: where a, simplex; b, rhomboidea; c, heteris and d, tenuis. Bar = 10 mm. (B) Leaf key that classifies intraspecific variants of *Capsella* according to the shape of their rosette leaves. Seven different leaf-shape variants are identified alongside the leaf criteria by which the different forms may be distinguished where solid line = 'yes' and dashed line = 'no'.

(Clark and Pregibon 1993). The process was carried out twice, initially with just the 53 accessions for which all functional traits were measured. This data-reduction step identified a subset of the functional traits which were important in the classification of the 53 accessions. This allowed the a classification tree to be constructed on a larger data set (128 individuals) for which these functional traits had been measured.

The functional relevance of the measured traits was assessed for the same 53 accessions using factor analysis. Factors were estimated by maximum likelihood and rotation based on variance maximisation. The number of

factors to include in the model was determined by sequentially fitting models with an increasing number of factors and testing the 'goodness-of-fit' at each stage by a generalised likelihood ratio test. Once identified, a comparison of the factors between leaf variants by multistratum analysis of variance was carried out as a more concise test of the functional differences between leaf variants. Finally, model based cluster analysis (Fraley and Raftery 2002a, 2002b) determined whether the plants might be partitioned into distinct classes on the basis of their functional traits alone, i.e. independently of their leaf form. The methods described above rely on an assumption that the data are normally distributed. Therefore, the variables were transformed prior to these analyses with the appropriate transformation being determined on examination of the Box–Cox profile likelihood function for the intercept-only model.

Simple Sequence Repeat Analysis

DNA extracted from two 0.8-mm-diameter *Capsella* leaf discs [Qiagen Ltd, Crawley, West Sussex, UK) plant (mini); #6910416] was processed for simple sequence repeat (SSR) analysis as described by Woodhead et al. (2003), using the 'touch-down' thermal profile: (1) 5 min at 94°C; (2) one cycle: 2, 0.5 min at each of 94, 58 and 72°C; (3) cycle 2 repeated seven times, with the annealing temperature being reduced by 1°C per cycle and (4) 24 cycles (as cycle 2) with the annealing temperature fixed at 58°C (PCR PE 9700 Thermal Cycler; PerkinElmer Inc., Beasonsfield Bucks). SSR primers were sourced from the peer-reviewed literature (Clauss et al. 2002, Kresovich et al. 1995, Szewc-McFadden et al. 1996). In total, 60 different primer pairs were trialled upon the DNA extracted from three individual plants (from different parental lines where possible), for each leaf class (see Table 1), plus positive (*Arabidopsis*) and negative controls.

Table 1. SSR primers, reported and detailed from *Arabidopsis* by Clauss et al. (2002), used to distinguish the seven *Capsella* variants, with corresponding range of allele sizes that were amplified by each primer pair for *Arabidopsis* and for *Capsella*.

		Size range (bp)	
Primer code	Primer nucleotide sequence	*Arapidopsis*	*Capsella*
ICE5.1	CTT GCA ACC GCC AAC TCA ATC G	166–241	234
ICE5.2	CCT GTC TCG CTC CCG CAC G		
ICE9.1	TTT CCC CAC ACA AAA TCT CC	110–159	104–106
ICE9.2	TTC CTT GCT CAA ATT GAA GG		
ICE7.1	TTC AAG GGC AGG ATC AAA AC	72–165	98–100
ICE7.2	GTC TCA CTG CTA TCG TCA CAG G		
ATTS0392.1	TTT GGA GTT AGA CAC GGA TCT G	134–171	181–184
ATTS0392.2	GTT GAT CGC AGC TTG ATA AGC		

Results

Leaf-type classification

In his definitive work from pedigreed crosses of accessions derived from many parts of the world, Shull (1909, 1914, 1918, 1923, 1929) concluded that the *Capsella* complex is comprised of 12 genetically distinct forms that belong to one of two phenotypically similar but sexually isolated species; namely, *C. rubella* ($2n = 2x = 16$) and *C. bursa-pastoris* ($2n = 4x = 32$); the former comprising four of the forms, and the latter eight intraspecific variants. The 12 'species' identified by Shull (1909) were categorised by their possession of four different leaf shapes: simplex, rhomboidea, heteris and tenuis (designated as a–d, respectively; Fig. 1A). However, the variety of leaf forms here extended beyond the four listed by Shull. A 'leaf-key', reliably distinguished seven different leaf types (Fig. 1B). Our leaf type I, encompasses Shull's 'simplex' and 'rhomboidea' forms, while our leaf types II, VI and VII correspond to the 'tenuis' shape, and leaf types III, IV and V, are most similar to Shull's 'heteris' (compare Fig. 1A, B).

Individuals possessing leaf type I were distinguished by their possession of a lamina that was (in relative terms) entire, that is lacking pronounced lobes, or leaflets. Where leaflets were apparent, they were not pronounced along the entire length of the leaf, nor did the leaf trough extend to touch the mid-rib (Fig. 1B). Leaf type II, possessed pronounced leaflets that ran the length of the leaf, and showed deep, round-bottomed leaf troughs. Leaf type VII, arose from the type II class, and occurred only rarely in a late-flowering form, that possessed a low number of rosette leaves, of two distinct forms that categorised it in a class of its own, between the generally earlier flowering leaf types I and II (Fig. 1B). Leaf type VI possessed leaflet edges that were serrated, while a band of leaf lamina ran along each side of the mid-rib; so the leaf trough did not contact the mid-rib. This contrasted with leaf type V, where the mid-rib was not subtended with lamina and whose leaflet serrations were more exaggerated than type VI. Leaf types III and IV could be distinguished by their lack of leaf lamina, the leaflets appearing long and narrow. Leaf type IV appeared darker green in colour (reflected in higher SPAD readings) and the upper edge of each leaflet closest to the midrib often possessed a distinct lobe (Fig. 1B).

Of the 53 maternal lines that were assessed, leaf types I and II accounted for ca. 83% of accessions in broadly similar numbers (23 and 21, respectively). Four accessions (7.5%) were leaf type III, while leaf type VI had two (3.8%) accessions and types IV, V and VII each possessed only one (1.9%). In keeping with our original

observations (described briefly in the Materials and methods section), the individuals that bore leaves resembling Shull's 'tenuis' and 'heteris' forms were found only infrequently. Furthermore, it was not possible to relate the seven different leaf types, or their relative numbers, to geographic distribution.

SSR analysis

In total, 60 different primer pairs were trialled upon the DNA extracted from three individual plants, of each leaf class (1–7). Tests of each PCR primers were therefore performed upon 21 samples plus positive (*Arabidopsis*/*Brassica*) and negative controls samples. For each leaf class, the individuals were selected from a different parental line wherever that was possible. While the individuals within leaf variants I and II could be sourced from different maternal lines, this was not possible for the remaining accessions. Two maternal sources were found for leaf variant VI and only one maternal source could be found for the remaining classes.

Four SSR primers pairs were able to distinguish between the 21 selected test samples (Fig. 2 and Table 1) on the basis of leaf class. These four primers were first described by Clauss et al. (2002).

Phenotypic characterisation

The mean values for each of the functional traits suggest that some functional differences exist between the seven *Capsella* variants (Table 2). This was confirmed by the multistratum analysis of variance that identified significant differences in functional traits relating to the timing of, and contribution to, reproduction and also differences in the amount of somatic tissue between leaf variants at the level of the individuals and maternal lines (Table 3).

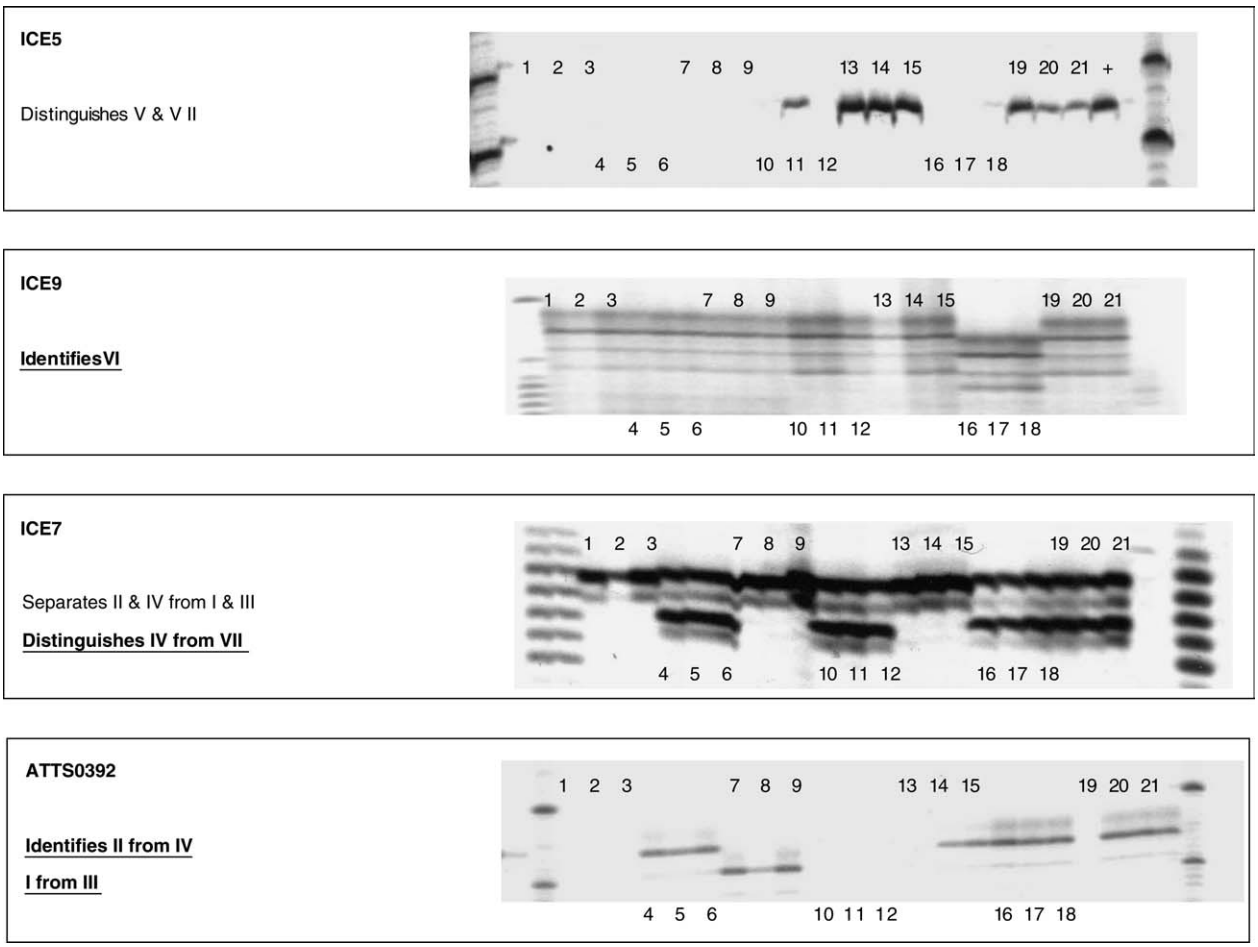

Fig. 2. Autoradiographs of PCR reactions products obtained using specific SSR primers to distinguish the *Capsella* variants. Numbers relate to the three different (replicate) plants of representative individuals of each leaf-type class; where lanes 1–3, 4–6, 7–9, 10–12, 13–15, 16–18 and 19–21 = leaf types I–VII, respectively.

Table 2. Mean values for the functional traits of seven *Capsella* variants. Sample size and standard error of the mean are in parentheses. SPAD, Single Photon Avalanche Diode; T_{50}, time for 50% of germination.

Trait	I	II	III	IV	V	VI	VII
Time to flower (days)	70.68 (118, 1.44)	74.32 (153, 1.24)	132.18 (11, 5.1)	124.6 (5, 5.44)	73 (8, 1.84)	87.55 (20, 3.02)	80.71 (7, 3.35)
Time to harvest (days)	144.63 (125, 1.79)	151.48 (170, 1.54)	192.82 (11, 2.49)	196.33 (6, 0.21)	143.25 (8, 3)	156.85 (20, 1.87)	153.43 (7, 5.02)
Reproductive duration (days)	74.52 (118, 1.4)	76.75 (153, 1.4)	60.6411 (4.89)	71.60 (5, 5.5)	70.25 (8, 2.51)	69.30 (20, 3.3)	72.71 (7, 7.32)
Rosette diameter (mm)	451.43 (125, 6.23)	468.54 (170, 5.64)	539.18 (11, 25.15)	502.33 (6, 11.42)	456.5 (8, 8.63)	482.1 (20, 16.56)	533.57 (7, 18.13)
Leaf number	47.79 (125, 2.65)	67.21 (170, 3.13)	91.73 (11, 8.97)	80.67 (6, 5.76)	190.13 (8, 14.55)	131.5 (20, 9.86)	119.29 (7, 16.08)
Stem number	9.82 (73, 0.51)	10.39 (119, 0.36)	6.55 (11, 1.58)	5.83 (6, 1.6)	15.86 (7, 1.74)	12.75 (20, 0.89)	11 (7, 2.01)
Stem weight (g)	28.72 (74, 1.85)	31.22 (106, 1.09)	22.48 (9, 2.79)	29.65 (5, 4.02)	32.64 (8, 2.24)	33.05 (15, 2.99)	45.64 (2, 22.3)
Total seed weight (g)	11.53 (51, 0.82)	12.13 (78, 0.68)	2.67 (8, 1.07)	4.39 (3, 1.66)	21.52 (5, 1.64)	10.16 (10, 0.58)	15.66 (2, 10.6)
Seed area (mm^2)	0.3610 (48, 0.0049)	0.3527 (77, 0.0044)	0.3885 (8, 0.0094)	0.3152 (3, 0.0036)	0.3881 (3, 0.0151)	0.3584 (7, 0.0144)	0.3246 (2, 0.0164)
Dormancy (%)	70.19 (48, 6.54)	67.67 (74, 4.06)	62.32 (8, 13.95)	61.28 (3, 9.59)	36.48 (3, 23.28)	56.38 (7, 11.70)	74.48 (2, 19.93)
Viability (days)	80.51 (48, 2.58)	81.82 (74, 1.75)	80.14 (8, 6.71)	80.07 (3, 3.56)	76.17 (3, 7.28)	82.46 (7, 5.32)	72.95 (2, 0.35)
T_{50} (days)	30.44 (48, 3.49)	37.07 (75, 2.97)	13.63 (8, 7.55)	25.67 (3, 15.62)	5.67 (3, 1.67)	25.86 (7, 10.09)	17 (2, 13)
Flush number	1.95 (44, 0.13)	1.96 (72, 0.08)	1.13 (8, 0.35)	2 (3, 0)	1.67 (3, 0.33)	2 (7, 0.22)	2.5 (2, 0.5)
SPAD	29.05 (50, 0.51)	29.38 (78, 0.51)	37.7 (9, 0.93)	42.27 (3, 1.16)	24.9 (3, 1.01)	28.66 (9, 0.64)	28.63 (3, 0.79)

Table 3. Results of the multistratum analysis of variance of the effect of *Capsella* variant on functional traits. SPAD, Single Photon Avalanche Diode; T_{50}, time for 50% of germination.

	Maternal line		Individual	
Trait	F	*P*	F	*P*
Time to flower (days)	3.7490	0.0041	7.8530	0.0000
Time to harvest (days)	3.6370	0.0049	6.5227	0.0003
Reproductive duration (days)	0.9593	0.4633	0.2444	0.8653
Rosette diameter (mm)	0.7230	0.6332	7.9174	0.0000
Leaf number	5.2999	0.0003	2.2418	0.0836
Stem number	3.2696	0.0097	0.5590	0.6481
Stem weight (g)	0.8591	0.5319	0.7444	0.5271
Total seed weight (g)	4.7623	0.0008	0.3903	0.7603
Seed area (mm^2)	1.3927	0.2379	3.5578	0.0173
Viability	0.0998	0.9960	1.0164	0.3893
T_{50} (days)	1.2140	0.3164	0.1500	0.9294
Flush number	1.8957	0.1019	1.0554	0.3722
SPAD	8.7512	0.0000	2.1590	0.0977
Dormancy	1.9773	0.0895	1.8001	0.1465

The classification trees elaborate the association between functional traits and the leaf variants further. Construction of the tree based on the 53 plants for which all traits had been measured used the parameters for time to flower, dormancy, reproduction duration, leaf number, seed area and rosette diameter—and achieved a misclassification rate of 21%. Selection of classification variables for the construction of the subsequent tree was limited to those traits identified in the former tree, allowing this analysis to be extended to data from 128 plants. The resulting tree successfully classified 73% of the plants on the basis of five of the functional traits (Fig. 3). Leaf variants III and IV were easily separated from other leaf forms by their late-flowering time (Fig. 3 and Table 2). These late-flowering types could be classified further on the basis of their relative seed areas with leaf variant IV plants producing smaller seeds than leaf variant III. A further distinction of leaf variants was also possible for the early flowering individuals, where leaf variant VI was separated from leaf variants I, II, V and VII on the basis of the relative number of rosette leaves (Fig. 3).

The factor analysis (Table 4 and Fig. 4) identified two factors (general likelihood ratio test: $\chi^2 = 47.58$, df = 34, $P = 0.061$) that were sufficient to describe a total of 45% of the variance of the 11 phenotypic traits that were measured. The loadings of each variable on the two factors are given (Table 4). The first factor has a high positive correlation with reproductive duration, flowering-stem number, total stem weight and total seed weight and a negative correlation with time to flowering and seed area. The second factor is positively correlated with the time to flowering, rosette diameter, rosette-leaf

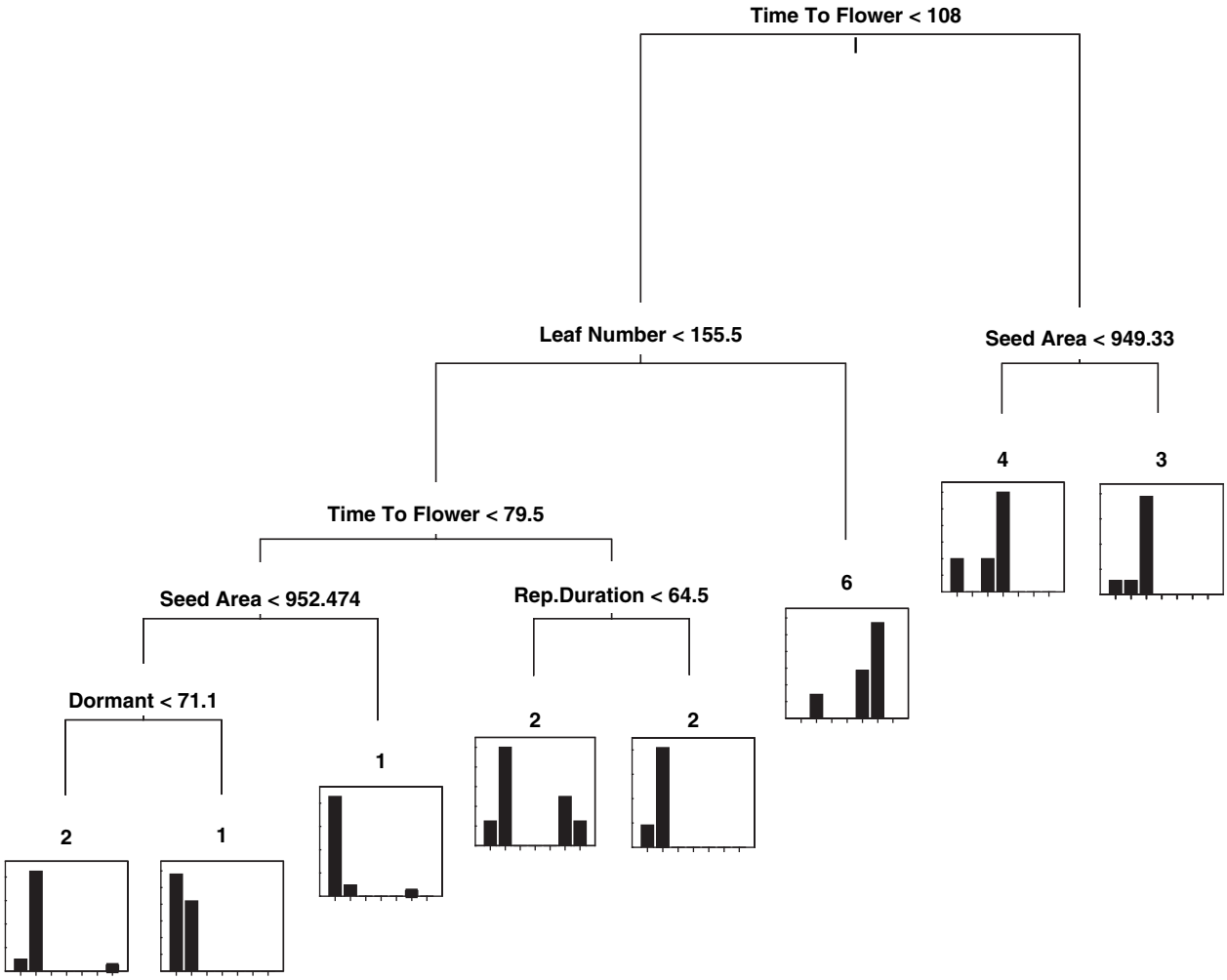

Fig. 3. Classification tree grown using a subset of functional traits for 128 plants (units in Table 1). The traits used in the classification are given at the internal nodes while the terminal nodes represent groups arising from the classification. These are labelled with the most frequent leaf variant in each of the classified groups with the relative frequency of the leaf variants presented in the histograms.

number and leaf maximum SPAD readings. These may be interpreted as '*reproductive output*' and '*somatic input*': plants demonstrating high *reproductive output* flower early and for longer possess a greater biomass of reproductive tissue yielding more but smaller seeds; plants with high *somatic input* combine a late time of flowering with large rosette diameter, rosette-leaf number and leaf maximum SPAD.

The factor analysis of the functional traits of *Capsella* was based on the individual plants without reference to leaf variant. However, by comparing the reproductive effort with somatic growth factors between leaf variants a further assessment of the functional differences can be made. In Fig. 4, the position occupied by individuals within the functional trait space shows that there is little evidence of functional similarity between individuals of the same leaf variant. Analysis of variance of these data identified a significant difference in the *somatic growth* factor between leaf variants at the maternal line strata ($df = 5$, $F = 4.47$, $P = 0.0045$). A subsequent multiple comparison test based on 5% family-wise error rate identified leaf variant 1 as having significantly greater scores for the *somatic growth* factor than leaf variants II or III. No differences were observed when comparing plants of different leaf variants within the same maternal lines or in the *reproductive effort* factor at either error strata.

The bayesian information criterion (BIC) (see Methods) was maximal for models with three clusters, which were either diagonal (traits uncorrelated) with varying volume and equal shape or diagonal with equal volume and equal shape (EEI); the difference in BIC between these models was just 2.25. However, the BIC did not differentiate

Table 4. The sum of squares, cumulative variance explained and loadings for a two-factor factor analysis model fitted to the *Capsella* functional trait data by maximum likelihood. SPAD, Single Photon Avalanche Diode.

	Factor 1	Factor 2
SS loadings	2.99	1.96
Cumulative variance	0.272	0.450
	Loadings	
1/Time to flower	0.767	−0.621
Reproductive duration	0.694	−0.163
Rosette diameter	0.140	0.567
Leaf number	0.046	0.629
Stem number	0.774	0.215
Stem weight	0.665	0.432
Total seed weight	0.720	−0.121
Seed area	−0.397	0.092
Seed viability	0.315	0.078
SPAD	−0.289	0.609
Dormant	0.015	−0.440

strongly between these and the other models, in terms of either the parameterisations of the covariance matrix or the number of clusters. Therefore, to test the sensitivity of the results to changes in the observations, cluster analysis was repeated on 1000 bootstrap samples of the data set. The results indicate that the optimal number of clusters in a model is affected by the parameterisation of the covariance matrix. However, for the majority of covariance parameterisations, models with three or four clusters most frequently provided the best-fit to the bootstrap data (data not shown) and over all parameterisations, 70% of the best-fit models had three or four clusters.

Parameter estimates for the three-cluster EEI model are given in Table 5. Examination of the cluster suggests that the observed *Capsella* plants can be classified as one of three types: cluster 1, late-flowering plants of intermediate size and low reproductive output; cluster 2, early flowering small plants with intermediate reproductive output; or cluster 3, large plants with intermediate time to flowering and high-reproductive output. However, these distinctions are not clear cut as the relatively large standard deviation for some traits makes clear. This is also evident in the lack of clear separation between observations of different classification when projected onto the various axes of the functional trait space. For example, Fig. 5 depicts the three-cluster classification from the EEI model projected onto the two-dimensional axes of leaf number and stem number, the combination for which cluster separation was most obvious.

A comparison of the classification of plants based on the three-cluster EEI model with that of leaf variant classification is presented in Table 5. There is no evidence of differential partitioning between the dominant leaf variants (I and II) (chi-square test: $\chi^2 = 0.1481$, df = 2, $P = 0.9286$). However, the few examples of the other variants appear to partition into clusters with leaf variants III and IV falling into cluster 1 and leaf variants V and VI into cluster 3 (Fig. 5).

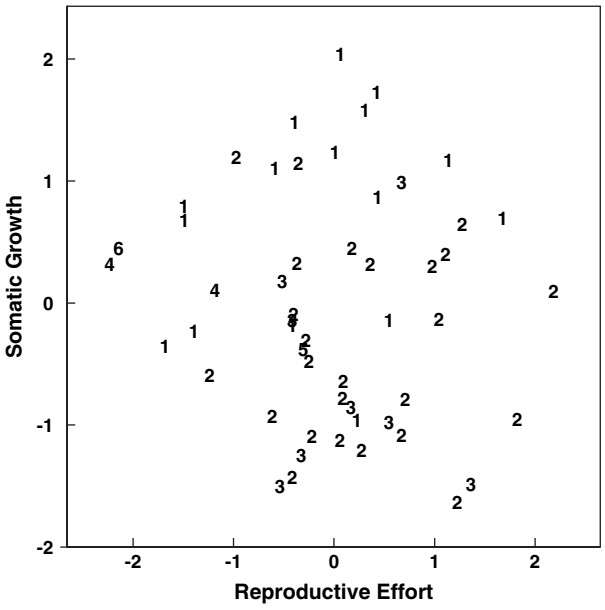

Fig. 4. Factor analysis scores from the two-factor model plotted to show the distribution of individuals of leaf variants I–VI in the two-dimensional trait space defined by the factors *reproductive effort* and *somatic growth*.

Discussion

The leaf-shape variants previously described (Aksoy et al. 1999, Shull 1909) were found in the preliminary screen, of which around 75% of the individuals possessed leaves of the forms 'simplex' or 'rhomboidea' (sensu Shull 1909; Fig. 1A). This initial classification guided the choice of accessions from which to take and germinate seed for the phenotypic characterisation, which then revealed seven leaf-shape variants, the most frequent two again corresponding to Shull's leaf forms 'a' and 'b' (Fig. 1A) and comprised 85% of the total individuals. While our leaf type I, encompasses Shull's 'simplex' and 'rhomboidea' forms (Fig. 1A; leaf-form types a *and* b) our other six leaf types are subdivisions of Shull's 'tenuis' (our leaf types II, VI and VII) and 'heteris' leaf forms (our leaf types III, IV and V). The types encompassed within Shull's 'heteris' and 'tenuis' forms are particularly broad, despite the low number of accessions for these leaf classes. Nevertheless, the first and second questions asked in the Introduction have been answered: the four leaf-shape variants are still readily found in the seedbank and all seven leaf-shape variants identified here were also distinguished by SSR

Table 5. Parameter estimates (mean and standard deviation) for the three-cluster Gaussian mixture model with diagonal covariance of equal volume and shape. sd, standard deviation; SPAD, Single Photon Avalanche Diode; T_{50}, time for 50% of germination.

Trait	1	2	3	SD
1/Time to flower	0.009 (111)	0.014 (71)	0.012 (83)	0.00000354
Reproductive duration	65.5	84.15	83.75	134.79
Rosette diameter	497.7	459.6	526.1	2557.78
Leaf number	6.46	5.04	7.04	0.45
Stem number	5.45	8.57	14.55	7.27
Stem weight	5.57	5.71	7.47	1.27
Total seed weight	3.87	10.87	12.15	18.11
Seed area	0.666462	0.66664269	0.66664344	9×10^{-12}
Viability	78.92	84.28	86.54	138.89
T_{50}	17.0	39.54	27.03	530.33
SPAD	34.38	26.88	30.65	23.30
Dormancy	1.10	1.25	0.91	0.15

markers (Fig. 2 and Table 2), confirming that the variants are genetically distinct. Moreover, subsequent experiments have confirmed that the leaf shapes characterised ex situ in these accessions are maintained in field (authors' data; not shown). Other authors have indicated that leaf shape is far more broad than depicted by Shull (1909). Neuffer and Bartelheim (1989) acknowledge that leaf shape could not be assessed for ca. 20 ± 5% of the population at each of 10 sites, and that for the individuals for whom no particular leaf shape could be attributed, 116 (ca. 78%) possessed either 'heteris-' or 'tenuis-' type leaf shapes. This supports the fact that leaf shape is broad,

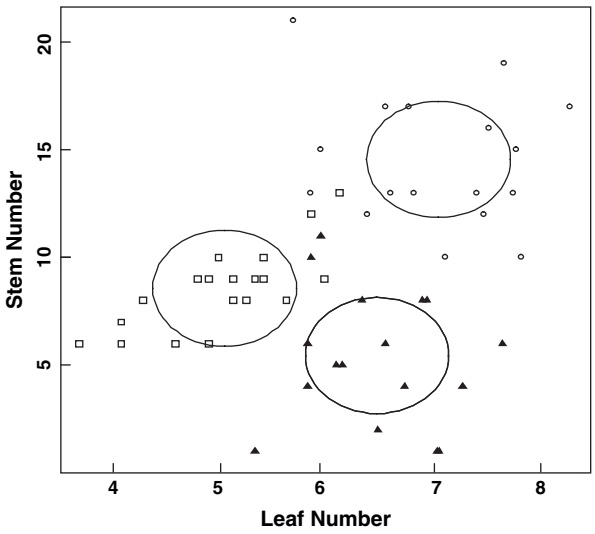

Fig. 5. A two-dimensional projection of the three-cluster classification for the Gaussian mixture model, with diagonal clusters of equal volume and shape onto the axes of leaf number and stem number, the combination for which, cluster separation was most obvious. Circles indicate the standard deviation of mixture components. Symbols identify plant classified into one of the three clusters.

and that this is especially true for the 'heteris' or 'tenuis' leaf classes.

Leaf-shape variants were not, however, uniquely associated with distinct values of the life-history traits. By cross-referencing the functional traits of the three clusters determined by cluster analysis (Table 5) with those of the leaf-shape variants (Table 2, Fig. 3) some similarity is evident: cluster 1 is broadly equivalent to variants III and IV, cluster 2 to variants I and II and cluster 3 to variants VI and VII (Table 5). Leaf shape cannot be relied upon as an attribute that is diagnostic for functional diversity by association with other traits; as leaf shape appears as only a partial indicator of functional diversity in its own right. Accessions as a whole displayed considerable individual trait variation that precluded the discrete partitioning of plants into functional classes either by leaf-shape or by functional traits alone. Nevertheless, the third question asked in the Introduction has been answered: intraspecific variation exists in functionally relevant traits that are likely to affect food-web resources.

Moreover, the individual accessions, rather than groups of functionally similar accessions, should be considered as the unit of biodiversity. Among the accessions, the factor analysis (Table 4) showed time to flowering was correlated variously with both reproductive and somatic traits but there was no simple trade-off between such traits. Early flowering types achieved high seed output because their reproduction was partly indeterminate in that they produced several flowering stems sequentially after flowering began. Reproductive duration was much more similar between the accessions than was time to flowering, so that the reproductive/somatic ratio was greater the earlier the plants started to flower. Even within the early flowerers, stem number ranged between 10 and 16 and was highly correlated

with seed mass, indicating some conservatism in seed mass per stem. If somatic and reproductive durations had been positively correlated, then early flowering variants would not have produced the most seed (which they did). In summary, an accession produced more seed than another if its first flowers came earlier and it produced subsequent flowering stems more rapidly. Time to flowering also had concomitant effects on inferred nitrogen, or associated chlorophyll; expressed through higher SPAD readings being recorded for the leaves of late flowerers. Presumably, this is because late-flowering individuals had longer to accumulate resources, and less reproductive tissue to which those resources could be translocated. It is likely that leaf-shape differences will impact upon carbon sequestration in preparation for flowering, and therefore will impact (differentially) upon fecundity, persistence and coexistence (Silvertown 2004), as there is a trade-off between photosynthetic CO_2 gain and water loss via transpiration (Farquhar and Sharkey 1982). Our future research will test the biological basis of potential differences in water use efficiency (Gutschick 1999, Guo et al. 2006), for the various *Capsella* leaf-shape forms.

Should early flowering, highly reproductive variants be expected to be most prevalent in arable fields? Autumn-sown crops have dominated in Britain since the 1980s. Disturbance typically comprises soil cultivation, and control of weeds in the autumn and (increasingly), variously in winter and spring too. The potential duration of most weed growth is generally set by the time between herbicide application in spring and harvest of the crop in late summer, a window of typically 100–120 days (Bohan et al. 2005, Champion et al. 2003). The early variants, flowering in around 70 days, and seeding well within 120 days, have the potential to be able to replenish the seedbank. However, the control of broadleaf weeds such as *Capsella* would have been more stringent in cereals, in which the number of applications and killing range of herbicides used in the main crops has increased since the 1980s (Marshall et al. 2003). They are more likely to have replenished the seedbank in the common broadleaf 'break' crops, such as oilseed rape, which are sown every 2–4 years. The high frequency of early flowering types with high reproductive capability is therefore consistent with a selective pressure imposed by frequent disturbance (Lee 2002, Palumbi 2001).

This notwithstanding, intensification had not reduced the *Capsella* phenotype to one early functional form. Late-flowering leaf type III variants arose from single accessions at five different sites and leaf type IV from another accession at one of those sites. The numbers of accessions found for each leaf type are too few to compare the presence of late-flowering variants with previous conditions, such as intensity of management at sites. However, opportunities must have existed for the late-flowering variants to persist or reseed. Persistence in the seedbank, without reseeding, from a time when cropping was less intense in the 1960s, appears unlikely. Decline rates of seed in the seedbank have seldom been measured for longer than 5–10 years but by extrapolating measured annual death rates of 50–75% a year for *C. bursa-pastoris* seed in soil (e.g. Roberts 1958, 1962), a typical *Capsella* seedbank of, say 1000 m^{-2}, 30 years ago, would not be readily detectable today by the sampling intensity used here. The late-flowering variants are more likely to have been maintained by reseeding in autumn-sown broadleaves on occasions when weed applications in the winter or spring were less than 100% effective. The late-flowering variants might also have been sustained by seed or pollen in-flow from plants in field margins or surrounding areas that are not exposed to frequent disturbance. However, the role of genetic isolation and 'genetic drift' must be assessed before a causal reason for the high abundance of short time-to-flowering ecotypes can be ascertained. Further testing as exemplified for *Arabidopsis* (Sharbel et al. 2000) would be informative. Sharbel et al. (2000) sampled *Arabidopsis* accessions from across Eurasia, including Mediterranean Pleistocene refugia, to assess the biogeographical and historical origins of functional diversity, and assess of the role of genetic drift. However, currently our findings do suggest that the increased prevalence of short time to flowering individuals capable of high fecundity in arable systems may be regarded at evidence of a strong selection pressure that may lead to an evolving trait; a common phenomenon in ecosystems that are strongly perturbed by human activities (Ghersa et al. 1994, Neuhauser et al. 2003).

The results open the way to examine functional biodiversity experimentally through traits rather than taxa. The approach could be extended, for instance, to compare common and declining arable species. The extensive seedbank studies between 2000 and 2003 (Champion et al. 2003, Heard et al. 2003) found that *C. bursa-pastoris* was the fourth most frequently found species after *Poa annua*, *Stellaria media* and *Urtica dioica* and just ahead of *Veronica persica* and *Sonchus oleraceus*. All these species are thought to be variable phenotypically in contrast to many declining annuals that have a limited flowering period. Given that the range of flowering-time variation, for instance, in *Capsella* is as wide as that among most annual, arable species, the accessions identified here provide initial plant material for testing the theoretical predictions of Pachepsky et al. (2001) that the properties shown by assemblages of species can be mimicked by assemblages of trait variants. Given further genetic characterisation to distinguish life-history traits rather than simply leaf-shape variants, the

effects of disturbance and intensity of management on the balance of *Capsella* variants could be examined across a wider range of environments or in reconstructed assemblages. As argued by Hawes et al. (2005), knowledge of *Arabidopsis* (Mitchell-Olds 2001) should accelerate a molecular dissection of the *Capsella* types.

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References

Aksoy A, Hale WHG, Dixon JM (1999) Towards a simplified taxonomy of *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae). Watsonia 22: 243–250

Bohan DA, Boffey CWH, Brooks DR, Clark SJ, Dewar AM, Firbank LG, Haughton AJ, Hawes C, Heard MS, May MJ, Osborne JL, Perry JN, Rothery P, Roy DB, Scott RJ, Squire GR, Woiwod IP, Champion GT (2005) Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. Philos Trans R Soc Lond B 272: 463–474

Champion GT, May MJ, Bennett S, Brooks DR, Clark SJ, Daniels RE, Firbank LG, Haughton AJ, Hawes C, Heard MS, Perry JN, Randle Z, Rossall M, Rothery P, Skellern MP, Scott RJ, Squire GR, Thomas MR (2003) Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philos Trans R Soc Lond B 358: 1801–1818

Charnov EV (1982) The Theory of Sex Allocation. Princeton University Press, Princeton, NJ

Clapham AR Tutin TG, Moore DM (1987) Flora of the British Isles, 3rd Edn. Cambridge University Press, Cambridge, UK

Clark LA, Pregibon D (1993) Tree-based models. In: Chamber JM, Hastie TJ (eds) Statistical Models. Chapman and Hall, London

Clauss MJ, Cobban H, Mitchell-olds T (2002) Cross-species microsatellite markers for elucidating population genetic structure in *Arabidopsis* and *Arabis* (Brassicaeae). Mol Ecol 11: 591–601

Diaz S, Cabido M (2001) Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 16: 646–655

Evans LT (1993) Crop evolution, adaptation and yield. Cambridge University Press, Cambridge, UK

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33: 317–345

Fraley C, Raftery AE (2002a) Model-based clustering, discriminant analysis, and density estimation. J Am Stat Assoc 97: 611–631

Fraley C, Raftery AE (2002b) MCLUST: software for model-based clustering, density estimation and discriminant analysis. Technical Report no. 329, Department of Statistics, University of Washington

Gallagher JN, Biscoe PV (1978) Radiation absorption, growth and yield of cereals. J Agric Sci 91: 47–60

Gaudet CL, Keddy PA (1988) A comparative approach to predict competitive ability from plant traits. Nature 334: 242–243

Ghersa CM, Roush ML, Radosevich SR, Cordray SM (1994) Co-evolution of agroecosystems and weed management: weed-management practices have become closely linked to social and economic, rather than biological factors. BioScience 44: 85–94

Guo S, Zhou Y, Song N, Shen Q (2006) Some physiological processes related to water use efficiency of higher plants. Agric Sci China 5: 403–411

Gutschick VP (1999) Biotic and abiotic consequences of differences in leaf structure. New phytol 143: 3–18

Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Bohan DA, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG, Squire GR (2003) Responses of plant and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically-modified herbicide-tolerant crops. Philos Trans R Soc Lond B 358: 1899–1913

Hawes C, Begg GS, Squire GR, Iannetta PPM (2005) Individuals as the basic accounting unit in studies of ecosystem function: functional diversity in *Capsella* (shepherd's purse). Oikos 109: 521–534

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish AM, Perry JN, Rothery P, Scott RJ, Skellern MP, Squire GR, Hill MO (2003) Non-crop plants in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Main effects of treatments. Philos Trans R Soc Lond B 358: 1819–1832

Hooper DU, Bignell DE, Brown VK, Brussard L, Dangerfield M, Wall DH, Wardle DA, Coleman DC, Giller KE, Lavelle P, Van der Putten W, Ruiter PC, Rusek J, Silver WL, Tiedje JM, Wolters V (2000) Interactions between aboveground and belowground diversity in terrestrial ecosystems: patterns, mechanisms and feedbacks. BioScience 50: 1049–1097

Koricheva J, Mulder CPH, Schmid B, Joshi J, Huss-Danell K (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. Oecologia 125: 271–282

Kresovich S, Szewc-McFadden AK, Bliek SM, McFerson JR (1995) Abundance and characterisation of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor Appl Genet 91: 206–211

Lavorel S, McIntyre S, Landsberg J, Forbes TDA (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. Tree 12: 474–478
Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17: 386–91
Loreau M (1998) Diversity and ecosystem functioning: a mechanistic model. Proc Natl Acad Sci USA 95: 5632–5626
Loreau M (2000) Diversity and ecosystem functioning: recent theoretical advances. Oikos 91: 3–17
Marshall EJP, Brown VK, Boatman ND, Lutman PJW, Squire GR, Ward LK (2003) The role of weeds in supporting biological diversity within crop fields. Weed Res 43: 77–89
Meikle RD (1977) Capsella, Flora of Cyprus. The Bentham-Moxon Trust, Royal Botanic Gardens, Kew, UK, pp 128–129
Mitchell-Olds T (2001) Arabidopsis thaliana and its wild relatives: a model system for ecology and evolution. Trends Ecol Evol 16: 693–700
Monteith JL (1977) Climate and the efficiency of crop production in Britain. Philos Trans R Soc Lond 281: 277–294
Neuffer B, Bartelheim S (1989) Genecology in *Capsella bursa-pastoris* from an altitudinal transect in the Alps. Oecologia 81: 521–527
Neuffer B, Eschner S (1995) Life-history traits and ploidy levels in the genus *Capsella* (Brassicaceae). Can J Bot 73: 1354–1365
Neuhauser C, Andow DA, Heimpel GE, May G, Shaw RG, Wagenius S (2003) Community genetics: expanding the synthesis of ecology and genetics. Ecology 84: 545–558
Norris RF, Kogan M (2000) Interactions between non-crop plants, arthropod herbivores, and their natural enemies in managed ecosystems. Weed Sci 48: 94–158
Pachepsky E, Crawford JW, Bown JL, Squire GR (2001) Towards a general theory of diversity. Nature 410: 923–926
Palumbi SR (2001) Humans as the world's greatest evolutionary force. Science 293: 1786–1790
Preston CD, Pearman DA, Dines TD (2002) New Atlas of the British and Irish Flora. Oxford University Press, Oxford
Roberts HA (1958) Studies on the weeds of vegetable crops. I. Initial effects of cropping on the weed seeds in the soil. J Ecol 46: 759–768
Roberts HA (1962) Studies on the weeds of vegetable crops. II. Effect of six years of cropping on the weed seeds in the soil. J Ecol 50: 803–813
Sharbel TF, Haubold B, Mitchell-Olds T (2000) Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonisation of Europe. Mol Ecol 9: 2109–2118
Shull GH (1909) *Bursa bursa-pastoris* and *Bursa heegeri* Biotypes. Carnegie Institution Publications, Washington, D.C. 112: pp 1–57
Shull GH (1914) Duplicate genes for capsule-form in Bursa bursa-pastoris. Z Indukt Abstamm Vererbungsl 12: 97–149
Shull GH (1918) The duplication of a leaf-lobe factor in shepherd's-purse. Mem Brooklyn Bot Gard 1: 427–443
Shull GH (1923) The species concept from the point of view of a geneticist. Am J Bot 10: 221–228
Shull GH (1929) Species hybridisation among old and new species of shepherd's purse. Proc Int Congr Plant Sci 1: 837–888
Siemann E, Tilman D, Haarstad J, Ritchie M (1998) Experimental tests of the dependence of arthropod diversity on plant diversity. Am Nat 152: 38–750
Silvertown J (2004) Plant coexistence and the niche. Trends Ecol Evol 19: 605–611
Squire GR, Brooks DR, Bohan DA, Champion GT, Daniels RE, Haughton AJ, Hawes C, Heard MS, Hill MO, May MJ, Osborne JL, Perry JN, Roy DB, Woiwod IP, Firbank LG (2003) On the rationale and interpretation of the farm-scale evaluations of genetically-modified herbicide-tolerant crops. Philos Trans R Soc Lond B 358: 1779–1800
Stinson CSA, Brown VK (1983) Seasonal changes in the architecture of natural plant communities and its relevance to insect herbivores. Oecologia 56: 67–69
Svensson S (1983) Chromosome numbers and morphology in the *Capsella bursa-pastoris* complex (Brassicaceae) in Greece. Willdenowia 13: 267–276
Szewc-McFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor Appl Genet 93: 534–538
Tutin TG, Burges NA, Chater AO (1993) Flora Europaea, 2nd Edn. Cambridge University Press, Cambridge, UK
Werker AR, Jaggard KW (1998) Dependence of sugar beet yield on light interception and evapotranspiration. Agric For Meteorol 89: 229–240
Woodhead M, Russell J, Squirrell J, Hollingsworth PM, Cardle L, Ramsay L, Gibby M, Powell W (2003) Development of EST-SSRs from the Alpine Lady-fern, *Athyrium distentifolium*. Mol Ecol Notes 3: 287–290
Young JEB, Griffin MJ, Alford DV, Ogilvy SE (2001) Reducing agrochemical use on the arable farm. Report of the TALISMAN and SCARAB projects. DEFRA, London

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