

# ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

## **Absence of phylogenetic signal in the niche structure of meadow plant communities**

by

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*Proc. R. Soc. B* ([doi:10.1098/rspb.2005.3288](https://doi.org/10.1098/rspb.2005.3288))

Electronic appendices are refereed with the text; however, no attempt is made to impose a uniform editorial style on the electronic appendices.

## Online Appendix

### *Species used in the study*

Table 1. List of species, mean  $\alpha$  niche metrics (SEV) at Tadham and Cricklade and Kew and Genbank accession numbers for *rbcL* sequences. Vouchers for species we collected ourselves are lodged at the Royal Botanic Gardens, Kew. Dashes in SEV columns indicate that the species was present in less than 50 samples from the site in question.

Species	Tadham		Cricklade		Accession #	
	SEV drought mean	SEV aeration mean	SEV drought mean	SEV aeration mean	Kew	Genbank
<i>Agrostis canina</i> sens. lat.	3.06	1.30	–	–	MWC10742	
<i>Agrostis capillaris</i>	3.67	1.32	7.94	1.24	MWC10743	AY395527
<i>Agrostis stolonifera</i>	2.40	1.81	4.24	3.51		
<i>Alopecurus geniculatus</i>	2.49	1.94	–	–		
<i>Alopecurus pratensis</i>	3.77	0.90	6.56	1.60	MWC10746	AY395528
<i>Arrhenatherum elatius</i>	–	–	7.82	1.02	MWC10747	AY395529
<i>Bellis perennis</i>	2.62	1.14	8.56	0.82	MWC10748	AY395530
<i>Bromus commutatus</i>	–	–	8.49	0.69		
<i>Bromus hordeaceus</i>	2.57	1.23	–	–	MWC10750	AY395531
<i>Caltha palustris</i>	1.03	2.58	–	–	MWC10752	AY395532
<i>Carex disticha</i>	1.80	2.04	–	–	MWC10754	
<i>Carex nigra</i>	2.09	1.83	5.07	3.04	MWC10755	AY395533
<i>Carex panicea</i>	2.32	1.56	–	–		
<i>Cirsium arvense</i>	3.72	1.00	–	–	MWC10761	AY395534
<i>Cirsium palustre</i>	3.17	1.04	–	–	MWC10760	
<i>Dactylis glomerata</i>	4.28	1.03	9.12	0.55	MWC10764	AY395535
<i>Eleocharis palustris</i>	1.68	2.67	–	–	–	<i>E. marginulata</i> :

Y13011

<i>Festuca pratensis</i>	2.68	1.41	6.58	2.07	MWC10770	AY395536
<i>Festuca rubra</i> agg.	3.28	1.32	7.74	1.14		
<i>Fritillaria meleagris</i>	–	–	8.07	1.11	MWC12064	AY395537
<i>Galium palustre</i> sens. lat.	1.60	2.74	–	–	MWC10771	<i>G. mollugo</i> : AY395538
<i>Glechoma hederacea</i>	3.99	1.26	–	–	–	Z37391
<i>Glyceria fluitans</i>	1.85	2.25	–	–	MWC10776	AY395539
<i>Heracleum sphondylium</i>	–	–	9.22	0.27	MWC10778	AY395540
<i>Hordeum secalinum</i>	–	–	7.13	1.46	MWC10779	AY395541
<i>Hypochaeris radicata</i>	3.78	1.56	–	–	MWC10780	AY395542
<i>Juncus articulatus</i>	2.90	1.36	–	–	MWC10781	AY395543
<i>Juncus effusus</i>	1.86	1.88	–	–	–	Genbank
<i>Lathyrus pratensis</i>	–	–	8.34	0.80	MWC10784	AY395544
<i>Leontodon autumnalis</i>	2.91	1.67	7.18	1.47	MWC10787	
<i>Leontodon hispidus</i>	2.40	1.55	8.38	0.96	MWC10785	AY395545
<i>Leontodon saxatilis</i>	–	–	8.88	0.59	MWC10786	
<i>Leucanthemum vulgare</i>	–	–	9.14	0.56	MWC10788	AY395546
<i>Lolium perenne perenne</i>	3.03	1.46	7.97	1.12	MWC10790	AY395547
<i>Luzula campestris</i>	3.66	0.99	–	–	MWC10792	AY395548
<i>Lychnis flos-cuculi</i>	1.57	2.01	–	–	MWC10794	AY395549
<i>Lysimachia nummularia</i>	2.51	1.56	–	–	MWC10795	AY395550
<i>Medicago lupulina</i>	–	–	9.93	0.13	MWC10797	AY395551
<i>Myosotis discolor</i>	2.83	1.41	–	–	MWC10798	AY395552
<i>Phleum pratense</i>	2.85	1.51	6.44	1.88	MWC10801	AY395553
<i>Poa pratensis</i> sens. lat.	3.05	1.43	–	–	MWC10802	
<i>Poa trivialis</i>	2.52	1.36	7.02	1.39	MWC10867	AY395554
<i>Persicaria amphibia</i>	2.00	1.94	–	–	MWC1080	AY395555

<i>Prunella vulgaris</i>	3.09	1.56	8.56	0.80	MWC10870	AY395556
<i>Ranunculus acris</i>	3.06	1.47	7.61	1.19	MWC10806	AY395557
<i>Rhinanthus minor</i>	–	–	7.83	1.29	MWC10807	AY395558
<i>Rumex acetosa</i>	3.11	1.32	7.46	1.22	MWC10809	AY395559
<i>Sanguisorba officinalis</i>	–	–	7.95	1.07	MWC10811	AY395560
<i>Senecio aquatica</i>	2.27	2.18	–	–	MWC10812	AY395561
<i>Taraxacum</i> agg.	2.94	1.43	8.03	1.00	MWC10815	AY395562
<i>Tragopogon pratensis</i>	–	–	9.16	0.38	MWC10816	AY395563
<i>Trifolium pratense</i>	3.15	1.40	7.80	1.13	MWC10818	AY395564
<i>Trifolium repens</i>	2.88	1.49	7.58	1.22	MWC10817	
<i>Trisetum flavescens</i>	–	–	9.41	0.40	MWC10879	AY395565
<i>Vicia cracca</i>	3.38	1.28	8.85	0.89	MWC10819	AY395566

### *DNA sequencing*

DNA sequences for *rbcL* were used because they give good resolution for analyses that contain species from many plant families. More rapidly evolving genes or regions would have been difficult to align with confidence. We recognize that the *rbcL* gene does not evolve rapidly enough to resolve most relationships between species within genera, but a preliminary analysis with a dataset restricted to one species per genus produced very similar results to those reported here for all 55 species, so we preferred to use all available data.

Total DNA was extracted from silica dried material using a CTAB method (Doyle and Doyle 1987). DNA was purified using CsCl/ethidium bromide (1.55mg/ml) density gradients in a Discovery 90 ultraspeed centrifuge (Sorvall). The ethidium bromide was removed from the DNA suspension with an equal volume of butan-1-ol (stored with x1

SSC) and dialysed once with Synthesis™ double distilled water (Milli-Q) for 4hr, and then twice with x1 TE buffer (4hr each).

Double stranded PCR amplification of *rbcL* was performed in an ABI thermal cycler. Pre-made 2.5mM MgCl<sub>2</sub> PCR Mastermix (ABgene), 14μM of forward and reverse primer, 1.0μl BSA (0.4% w/v), and between 50-100ng of total DNA, in 50μl reaction volumes. Thermal cycler conditions were (1) 96°C, 1min; (2) 96°C, 1min, (3) 48°C, 30sec, (4) 72°C, 1min; cycle (2)-(4) was repeated for 28 cycles, (5) 72°C, 7min; (6) 4°C. Products were cleaned using QIAquick PCR Purification Kit (Qiagen).

Dicot. genera were amplified using:

1F - 5' ATG TCA CCA CAA ACA GAA AC 3'

724Rd - 5' TCG CAT GTA CCT GCA GTA GC 3'

636F - 5' GCG TTG GAG AGA TCG TTT CT 3'

1460R - 5' TCC TTT TAG TAA AAG ATT GGG CCG AG 3'

Monocot. genera were amplified with:

1F (as above)

724Rm - 5' TCG CAT GTA CCY GCA GTT GC 3'

627F - 5' CAT TTA TGC GCT GGA GAG ACC 3'

1360R - 5' CTT CAC AAG CAG CAG CTA GTT C 3'

For *Carex* and *Juncus* the 1FA primer was used instead of the 1F:

1FA - 5' ATG TCA CCA CAA ACA GAG AC 3'

The cycle sequencing reactions were performed using the dideoxy chain termination method (5). The reactions were carried out in 5.25μl reaction volumes containing 2.0μl Bigdye® Terminator v3.1 (Applied Biosystems, ABI cycle sequencer kit) 10-20ng of

template DNA, 3.5 $\mu$ M, ). Reactions were subjected to conditions in the same thermal cyclor according to ABI instructions, but for 26 cycles, not 25. Cycle sequence products were purified according to ABI ethanol precipitation protocol and analyzed using a 3100 automated genetic analyzer (Applied). The raw ABI sequence files were assembled and edited using Sequencher version 4.1.2.

*rbcL* sequences for two species (*Glechoma hederacea* and *Eleocharis marginulata*), as well as that for *Asplenium trichomanes* used for rooting the tree, were obtained from Genbank rather than our own study. In addition, for two genera, the *rbcL* sequence was for a different species than the niche data, because a sequence was not available for the exact species in question (*Galium mollugo* with *G. palustre* niche data; *Eleocharis marginulata* with *E. palustris* niche data).

#### *Reference*

Doyle, J. J. & Doyle, J. L.. 1987 A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* **19**, 11-15.

#### *Phylogeny*

Appendix Fig.1. A phylogenetic tree for 55 species present in the two meadow communities. Branch lengths are based upon the ML estimate of the number of substitutions per site (note scale). Note that not all species shown were present in both communities.



