

These are electronic appendices to the paper by Bidartondo *et al.* 2004 Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proc. R. Soc. Lond. B* **271**, 1799–1806. (doi 10.1098/rspb.2004.2807)

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

Electronic Appendix A. Plants sampled for isotope ratio mass spectrometric analysis. L corresponds to Ellenberg's light indicator values. Functional group abbreviations: ECM, ectomycorrhizal; AM, arbuscular mycorrhizal; NM, non-mycorrhizal; FIX, N₂-fixer.

Site	Orchid	n	L	Non-orchid	Abbreviation	n	L	Functional group
1	<i>Cephalanthera damasonium</i>	4	3	<i>Fagus sylvatica</i>	<i>F.s.</i>	4	3	ECM
1	<i>Cephalanthera rubra</i>	4	4	<i>Helianthemum nummularium</i>	<i>H.n.</i>	1	7	ECM
				<i>Anthericum ramosum</i>	<i>A.r.</i>	4	7	AM/NM
				<i>Euphorbia cyparissias</i>	<i>E.c.</i>	4	8	AM/NM
				<i>Galium verum</i>	<i>G.v.</i>	4	7	AM/NM
				<i>Polygala chamaebuxus</i>	<i>P.c.</i>	3	6	AM/NM
				<i>Polygonatum multiflorum</i>	<i>P.m.</i>	1	3	AM/NM
1	<i>Epipactis atrorubens</i>	4	6	<i>Fagus sylvatica</i>	<i>F.s.</i>	3	3	ECM
				<i>Coronilla varia</i>	<i>C.v.</i>	4	7	AM/FIX
				<i>Anthericum ramosum</i>	<i>A.r.</i>	4	7	AM/NM
				<i>Galium verum</i>	<i>G.v.</i>	4	7	AM/NM
1	<i>Platanthera chlorantha</i>	4	6	<i>Helianthemum nummularium</i>	<i>H.n.</i>	1	7	ECM
				<i>Anthericum ramosum</i>	<i>A.r.</i>	2	7	AM/NM
				<i>Euphorbia cyparissias</i>	<i>E.c.</i>	2	8	AM/NM
				<i>Galium verum</i>	<i>G.v.</i>	2	7	AM/NM
				<i>Polygala chamaebuxus</i>	<i>P.c.</i>	1	6	AM/NM
				<i>Polygonatum multiflorum</i>	<i>P.m.</i>	1	3	AM/NM
1	<i>Neottia nidus-avis</i>	4	2					
2	<i>Epipactis helleborine</i>	4	3	<i>Fagus sylvatica</i>	<i>F.s.</i>	4	3	ECM
				<i>Lathyrus vernus</i>	<i>La.v.</i>	4	4	AM/FIX
				<i>Aegopodium podagraria</i>	<i>A.p.</i>	1	5	AM/NM
				<i>Convallaria majalis</i>	<i>C.m.</i>	4	5	AM/NM
				<i>Fragaria vesca</i>	<i>F.v.</i>	1	7	AM/NM
				<i>Hedera helix</i>	<i>H.h.</i>	2	4	AM/NM
3	<i>Epipactis distans</i>	4	3	<i>Quercus robur</i>	<i>Q.r.</i>	1	7	ECM
				<i>Anthyllis vulneraria</i>	<i>A.v.</i>	1	8	AM/FIX
				<i>Lotus corniculatus</i>	<i>L.c.</i>	2	7	AM/FIX
				<i>Ononis repens</i>	<i>O.r.</i>	1	8	AM/FIX
				<i>Euphorbia cyparissias</i>	<i>E.c.</i>	4	8	AM/NM
				<i>Juniperus communis</i>	<i>J.c.</i>	2	9	AM/NM
				<i>Sesleria albicans</i>	<i>S.a.</i>	4	7	AM/NM
4	<i>Epipactis palustris</i>	4	8	<i>Trifolium pratense</i>	<i>T.p.</i>	4	7	AM/FIX
4	<i>Dactylorhiza majalis</i>	4	8	<i>Briza media</i>	<i>B.m.</i>	4	8	AM/NM
				<i>Colchicum autumnale</i>	<i>C.a.</i>	4	6	AM/NM
				<i>Lysimachia vulgaris</i>	<i>L.v.</i>	4	6	AM/NM

Electronic Appendix B. PCR primers used in this study.

Name	Sequence 3'-5'	Target	Specificity	Reference
ITS1	tccgtaggtgaacctgcgg	nrITS	eukaryotic	White <i>et al.</i> 1990
ITS1F	cttggtcatttagaggaagtaa	nrITS	fungal	Gardes & Bruns 1993
ITS4	tcctccgcttattgatatgc	nrITS	eukaryotic	White <i>et al.</i> 1990
ITS4B	caggagacttgtagacgggccag	nrITS	basidiomycete	Gardes & Bruns 1993
ITS4-Tul	ccgccagattcacacattga	nrITS	tulasnelloid	Taylor 1997
TW14	gctatcctgagggaaacttc	nrLSU	eukaryotic	unpublished
ML5	ctcgcaaattatcctcataag	mtLSU	fungal	White <i>et al.</i> 1990
ML6	cagtagaagctgcatagggtc	mtLSU	fungal	White <i>et al.</i> 1990
NS5	aactaaaggaattgacggaag	nrSSU	eukaryotic	White <i>et al.</i> 1990
cML5.5	tagcggcttaactatgagg	mtLSU	fungal	White <i>et al.</i> 1990
MLin3	cgacacaggttcgtaggtag	mtLSU	fungal	White <i>et al.</i> 1990

References

- Gardes, M. & Bruns, T. D. 1993 ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **2**, 113-118.
- Taylor, D. L. 1997 The evolution of myco-heterotrophy and specificity in some North American orchids. Ph. D. dissertation, University of California at Berkeley.
- White, T. J., Bruns, T. D., Lee, S. & Taylor, J. W. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications* (eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White), pp. 315-322. San Diego: Academic Press.

Electronic Appendix C. Molecular characterization of mycorrhizal fungi from German orchids. Only known mycorrhizal lineages (orchid, ectomycorrhizal and/or ericoid) are listed. Obligately ectomycorrhizal lineages are shown in bold. Root numbers are not listed for orchid roots where pelotons (i.e., orchid mycorrhizas) were not observed. The results from 2-4 sections individually analyzed for each root have been combined.

Site	Host species	Plant	Root	Pelotons	ITS [‡]	mtLSU [§]	ITS1/ITS4-Tul	ITS1F/TW14
1	<i>Cephalanthera damasonium</i>	1		no	n.d.	n.d.	n.d.	n.d.
1	<i>Cephalanthera damasonium</i>	2	4080	yes	<i>Tomentella</i>	theleporoid	-	n.d.
1	<i>Cephalanthera damasonium</i>	2	4081	yes	<i>Tomentella</i>	theleporoid	-	n.d.
1	<i>Cephalanthera damasonium</i>	2	4082	yes	<i>Tomentella</i>	theleporoid	-	<i>Tomentella</i>
1	<i>Cephalanthera damasonium</i>	3	4083	yes	<i>Thelephora</i>	-	-	n.d.
1	<i>Cephalanthera damasonium</i>	3	4084	yes	<i>Inocybe</i>	-	-	n.d.
1	<i>Cephalanthera damasonium</i>	3	4085	yes	<i>Cortinarius</i>	cortinarioid	-	<i>Cortinarius</i> [†]
1	<i>Cephalanthera damasonium</i>	4	4086	yes	<i>Hymenogaster</i>	-	-	n.d.
1	<i>Cephalanthera damasonium</i>	4	4087	yes	<i>Hymenogaster</i>	-	-	n.d.
1	<i>Cephalanthera damasonium</i>	4	4088	yes	<i>Hymenogaster</i>	-	-	<i>Hymenogaster</i>
1	<i>Cephalanthera rubra</i>	1	4089	yes	<i>Tomentella</i>	-	-	n.d.
1	<i>Cephalanthera rubra</i>	2	4090	yes	<i>Tomentella</i>	theleporoid	-	n.d.
1	<i>Cephalanthera rubra</i>	3	4091	yes	<i>Tomentella</i>	-	-	n.d.
1	<i>Cephalanthera rubra</i>	4	4092	yes	<i>Leptodontidium</i> <i>Phialophora</i> *	theleporoid	-	n.d.
1	<i>Cephalanthera rubra</i>	4	4093	yes	-	theleporoid	-	n.d.
1	<i>Epipactis atrorubens</i>	1	4094	yes	<i>Inocybe</i>	-	-	<i>Inocybe</i> [†]
1	<i>Epipactis atrorubens</i>	1	4095	yes	<i>sebacinoid</i> *	-	-	n.d.
1	<i>Epipactis atrorubens</i>	2		no	n.d.	n.d.	n.d.	n.d.
1	<i>Epipactis atrorubens</i>	3	4100	yes	<i>Wilcoxina</i>	-	-	n.d.
1	<i>Epipactis atrorubens</i>	3	4101	yes	<i>Wilcoxina</i>	-	-	n.d.
1	<i>Epipactis atrorubens</i>	3	4102	yes	<i>Phialophora</i> *	<i>Tulasnella</i> *	<i>tulasnelloid</i> *	n.d.
1	<i>Epipactis atrorubens</i>	4	4104	yes	<i>Leptodontidium</i> <i>Tuber</i>	-	-	n.d.
1	<i>Platanthera chlorantha</i>	1	4096	yes	-	<i>Tulasnella</i> *	<i>tulasnelloid</i> *	n.d.
1	<i>Platanthera chlorantha</i>	1	4097	yes	<i>Phialophora</i> *	<i>Tulasnella</i> *	<i>tulasnelloid</i> *	n.d.
1	<i>Platanthera chlorantha</i>	2	4098	yes	-	<i>Tulasnella</i> *	<i>tulasnelloid</i> *	n.d.
1	<i>Platanthera chlorantha</i>	2	4099	yes	<i>Leptodontidium</i> <i>Phialophora</i> <i>Ceratobasidium</i>	-	-	n.d.
2	<i>Epipactis helleborine</i>	1	4116	yes	<i>Tuber</i>	-	-	<i>Tuber</i>
2	<i>Epipactis helleborine</i>	1	4117	yes	<i>Ceratobasidium</i>	-	-	n.d.
2	<i>Epipactis helleborine</i>	2	4118	yes	<i>Wilcoxina</i>	-	-	n.d.
2	<i>Epipactis helleborine</i>	2	4119	yes	<i>Wilcoxina</i>	-	-	n.d.
2	<i>Epipactis helleborine</i>	3		no	n.d.	n.d.	n.d.	n.d.
2	<i>Epipactis helleborine</i>	4	4121	yes	<i>Sebacinaceae</i> *	-	-	n.d.
3	<i>Pinus sylvestris</i>		4109	n/a	corticoid*	-	n.d.	n.d.
3	<i>Pinus sylvestris</i>		4110	n/a	corticoid*	-	n.d.	n.d.
3	<i>Pinus sylvestris</i>		4111	n/a	<i>Rhizopogon</i>	suilloid	n.d.	n.d.
3	<i>Pinus sylvestris</i>		4112	n/a	<i>Inocybe</i>	cortinarioid	n.d.	n.d.
3	<i>Pinus sylvestris</i>		4113	n/a	<i>Wilcoxina</i>	-	n.d.	n.d.
3	<i>Epipactis distans</i>	1	4105	yes	<i>Wilcoxina</i>	-	-	n.d.
3	<i>Epipactis distans</i>	1	4106	yes	<i>Wilcoxina</i>	-	-	n.d.
3	<i>Epipactis distans</i>	1	4107	yes	<i>Wilcoxina</i>	-	-	n.d.
3	<i>Epipactis distans</i>	1	4108	yes	<i>Wilcoxina</i>	-	-	n.d.
3	<i>Epipactis distans</i>	2	4114	yes	<i>Wilcoxina</i>	-	-	n.d.
3	<i>Epipactis distans</i>	2	4115	yes	<i>Wilcoxina</i>	-	-	n.d.

4	<i>Dactylorhiza majalis</i>	1	4064	yes	<i>Tulasnella*</i>	<i>Tulasnella*</i>	-	n.d.
4	<i>Dactylorhiza majalis</i>	1	4065	yes	<i>Tulasnella*</i>	<i>Tulasnella*</i>	-	<i>Tulasnella*</i>
4	<i>Dactylorhiza majalis</i>	2	4066	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Dactylorhiza majalis</i>	2	4067	yes	<i>Tulasnella*</i>	<i>Tulasnella*</i>	-	n.d.
					<i>Ceratobasidium</i>			
4	<i>Dactylorhiza majalis</i>	3	4068	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Dactylorhiza majalis</i>	3	4069	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Dactylorhiza majalis</i>	4	4070	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Dactylorhiza majalis</i>	4	4071	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	1	4072	yes	<i>Leptodontidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	1	4073	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	2	4074	yes	<i>Ceratobasidium</i>	-	-	<i>Ceratobasidium</i>
4	<i>Epipactis palustris</i>	2	4075	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	3	4076	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	3	4077	yes	<i>Ceratobasidium</i>	-	-	n.d.
4	<i>Epipactis palustris</i>	4	4078	yes	<i>sebacinoid*</i>	-	-	<i>Sebacina*</i>
4	<i>Epipactis palustris</i>	4	4079	yes	<i>Ceratobasidium</i>	-	-	n.d.

Notes:

* Lineages known to contain some ectomycorrhizal strains.

‡ Results from ITS1F/ITS4 and ITS1F/4B have been combined.

§ Results from ML5/ML6, cML5.5/ML6 and MLin3/ML6 have been combined. The latter amplified an orchid mitochondrial fragment.

† Partial sequences were obtained that sufficed for identification purposes.

Electronic Appendix D. Molecular characterization of fungi from three additional *Epipactis* species sampled outside Germany. All fungal identifications are based on nrITS sequence data obtained from individual root sections containing fungal pelotons. Obligately ectomycorrhizal fungal lineages are shown in bold.

Host species	Location	State/Country	Roots	Sections	Fungi (No. sections where detected)
<i>Epipactis dunensis</i>	Anglesey	Wales	1	7062	Tuber (1)
<i>Epipactis dunensis</i>	Anglesey	Wales	6	7066-71	Inocybe (1), Pezizales (1), Tuber (1)
<i>Epipactis dunensis</i>	Anglesey	Wales	4	7073-76	Pezizales (1), Tuber (3)
<i>Epipactis dunensis</i>	Anglesey	Wales	1	7078	Tuber (1)
<i>Epipactis dunensis</i>	Anglesey	Wales	4	7082-85	Pezizales (3)
<i>Epipactis dunensis</i>	Anglesey	Wales	3	7087-89	Tuber (2)
<i>Epipactis dunensis</i>	Anglesey	Wales	3	6043-45	Tuber (3)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	2	3075-82	Pyronemataceae (4)
<i>Epipactis gigantea</i>	Angelo Reserve	California	1	3084-87	Sebacinaceae (3)
<i>Epipactis gigantea</i>	Angelo Reserve	California	2	3089-93	<i>Piriformospora</i> (3)
<i>Epipactis gigantea</i>	Angelo Reserve	California	1	3094-95	Pyronemataceae (1)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	2	3096-99	<i>Tulasnella</i> (2)
<i>Epipactis gigantea</i>	Angelo Reserve	California	3	4000-03	Pyronemataceae (4)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	3	4006-10	Pyronemataceae (3)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	2	4018-22	<i>Tulasnella</i> (3)†
<i>Epipactis gigantea</i>	Angelo Reserve	California	1	4023-24	Pyronemataceae (2)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	3	4029-37	<i>Piriformospora</i> (7), Pyronemataceae (2)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	2	4039-42	<i>Ceratobasidium</i> (2), <i>Tulasnella</i> (1)†
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	2	4043-45	<i>Thanatephorus</i> (2)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	1	4047-48	Pyronemataceae (2)
<i>Epipactis gigantea</i> [*]	Angelo Reserve	California	1	4049-52	<i>Tulasnella</i> (3)
<i>Epipactis gigantea</i> [§]	Angelo Reserve	California	3	4053-56	<i>Ceratobasidium</i> (2)
<i>Epipactis gigantea</i> [§]	Crescent City	California	3	6008-10	<i>Tulasnella</i> (2)†
<i>Epipactis gigantea</i> [§]	Crescent City	California	2	6011-12	<i>Piriformospora</i> (2)
<i>Epipactis gigantea</i> [§]	Sharp's Creek	Oregon	2	6137-38	Pezizales (2)
<i>Epipactis gigantea</i> [§]	Sharp's Creek	Oregon	4	6139-42	<i>Ceratobasidium</i> (3)
<i>Epipactis helleborine</i>	UCBG	California	1	1.1.a-d	Pyronemataceae (4)
<i>Epipactis helleborine</i>	UCB	California	2	e.y.5.1-5.2	Tuber (2)
<i>Epipactis helleborine</i>	UCB	California	2	e.p.y.A, e.b.C	Tuber (1), Wilcoxina (1)
<i>Epipactis helleborine</i>	UCB	California	3	A2-A5	Pyronemataceae (4)
<i>Epipactis helleborine</i>	UCB	California	3	B1-B3	Piloderma (1), Pyronemataceae (2)
<i>Epipactis helleborine</i>	UCB	California	1	E3	Pyronemataceae (1)
<i>Epipactis helleborine</i>	UCB	California	1	D2	Pyronemataceae (1)
<i>Epipactis helleborine</i>	UCB	California	2	F1, F2	Pyronemataceae (2)
<i>Epipactis helleborine</i>	UCBG	California	2	15.2-15.3	Tuber (2)
<i>Epipactis helleborine</i>	UCBG	California	3	12.1-12.3	Tuber (2)
<i>Epipactis helleborine</i>	UCBG	California	1	1.2.a, 1.2.b	Pyronemataceae (2)
<i>Epipactis helleborine</i>	UCBG	California	1	2.1.a	Pyronemataceae (1)
<i>Epipactis helleborine</i>	UCBG	California	2	8.3.a, b-d	Tuber (2), Wilcoxina (1)
<i>Epipactis helleborine</i>	UCBG	California	1	4.2.b	Pyronemataceae (1)

<i>Epipactis helleborine</i>	Heiburg Forest	New York	1	6123	Tuber (1)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	1	6124	<i>Phialophora</i> (1)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	2	6125-27	Tuber (3)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	1	6128-29	Tuber (2)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	1	6130	Tuber (1)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	1	6131	<i>Leptodontidium</i> (1)
<i>Epipactis helleborine</i>	Heiburg Forest	New York	2	6132-35	Tuber (2)
<i>Epipactis helleborine</i>	NYBG	New York	4	6024-28	Pezizales (4)
<i>Epipactis helleborine</i>	NYBG	New York	4	6029-32	Pezizales (4)
<i>Epipactis helleborine</i>	Syracuse	New York	4	6091-96	<i>Leptodontidium</i> (2), Tuber (3)
<i>Epipactis helleborine</i>	Montréal Bot. Gard.	Québec	2	6098-99	Tuber (2)
<i>Epipactis helleborine</i>	Cambridge	Massachusetts	3	6005-07	Tuber (2)
<i>Epipactis helleborine</i> [‡]	Cambridge	Massachusetts	3	6000-04	Tuber (4)
<i>Epipactis helleborine</i>	Warburg Reserve	England	4	7001-04	<i>Leptodontidium</i> (3)
<i>Epipactis helleborine</i>	Warburg Reserve	England	2	7023-24	<i>Ceratobasidium</i> (1)

Notes:

* Non-photosynthetic underground seedling.

‡ Non-photosynthetic mature plant.

§ *Epipactis gigantea* plants adjacent to young, or rarely under mature, *Alnus rubra*. (All *E. dunensis* were under mature *Pinus sylvestris* and all *E. helleborine* were under mature *Quercus* spp., *Pinus* spp. or *Carpinus* spp.).

† DNA sequences obtained from PCR products generated with the primer combination ITS1/ITS4Tul. All others were generated with ITS1F/ITS4, except 1.1.a-d which was generated with NS5/ITS4.

Abbreviations: NYBG, New York Botanical Garden; UCBG, University of California Botanical Garden; UCB, University of California at Berkeley.