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Survey of the nothogenus ×*Elyhordeum*, with the description of a new nothospecies

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Abstract

The occurrences in Denmark, Britain and Ireland of ×*Elyhordeum langei* (*Elymus repens* × *Hordeum secalinum*) are documented, and the variation in this hybrid is described. The French hybrid ×*E. rouxii* is also briefly covered. The discovery, distribution and characters of a new hybrid, *E. caninus* × *H. secalinum*, first found in North Lincolnshire, England, in 2014, are described and discussed. Cytological and molecular studies are included. The latter is described as ×*E. kirbyi* M.P.Wilcox, **hybr. nov.**

Keywords: *Elymus*, *Hordeum;* intergeneric hybrid; ITS sequencing; chromosome number; unreduced gametes; chloroplast DNA.

Introduction

Poaceae tribe Triticeae Dumort. (syn. Hordeeae) has been recognised in more or less its present circumscription for many years (Hubbard, 1948; Soreng et al., 2017); in the past 70 years it has not been subject to the multiple rearrangements that have beset many other tribes, notably the Poeae (syn. Festuceae). It contains the important cereals wheat (*Triticum* L.), barley (*Hordeum* L.) and rye (*Secale* L.), as well as the wild grasses variously known as wheatgrasses or couchgrasses that have until quite recently been mainly placed in the genus *Agropyron* Gaertn. This paper concerns natural intergeneric hybrids between *Hordeum* and the couchgrasses.

In the British literature our four species of couchgrass were retained in *Agropyron* until Melderis (1978) segregated them into the genus *Elymus* L. Two of the species, *E. repens* (L.) Gould (Common Couch) (Figs. 1A-C) and *E. caninus* (L.) L. (Bearded Couch) (Fig. 1D) have retained their specific epithets during several taxonomic realignments, but the other two have accumulated multiple synonyms, partly due to the rules of nomenclature and partly because of changing taxonomic opinions. The Sea Couch was known as *Agropyron pungens* (Pers.) Roem. & Schult. by Hubbard (1954) and Tutin (1962), *Elymus pycnanthus* (Godron) Melderis by Melderis (1978), *Elytrigia atherica* (Link) Kerguélen by Stace (2010) and Stace et al. (2015), *Elytrigia acuta* (DC.) Tzvelev by Tison & de Foucault (2014), *Thinopyrum acutum* (DC.) Banfi by Banfi (2018), and *Elymus athericus* (Link) Kerguélen by Cope & Gray (2009), Stace (2019) and Duistermaat (2020). The classification of the fourth species, Sand Couch, is complicated by the fact that there has been disagreement as to whether the Mediterranean hexaploid and Atlantic tetraploid populations should be placed in the same species (as separate subspecies) or treated as separate species. Our taxon, the tetraploid, was known as *Agropyron junceiforme* (Á. & D. Löve) Á. & D. Löve by Hubbard (1954) and Tutin (1962), *Elymus farctus* (Viv.) Runemark ex Melderis subsp. *boreali-atlanticus* (Simonet & Guin.) Melderis by Melderis (1978), Cope & Gray (2009) and Duistermaat (2020), *Elytrigia juncea* (L.) Nevski subsp. *boreoatlantica* (Simonet & Guin.) Hyl. by Stace (2010), Tison & de Foucault (2014) and Stace et al. (2015), *Thinopyrum junceiforme* (Á. & D. Löve) Á. Löve by Banfi (2018), and *Elymus junceiformis* (Á. & D. Löve) Hand & Buttler by Stace (2019).

It is of course important to understand such a wide range of opinions when consulting the extensive literature, but an appraisal of their relative merits is not relevant here. Nor do the numerous wild interspecific hybrids within the genus *Elymus* concern the present study. To simplify matters for present purposes we recognise the two chromosome races (Mediterranean and Atlantic) of the Sand Couch as separate species, and place all four British species of couchgrass in the genus *Elymus* as *E. caninus*, *E. repens*, *E. athericus* and *E. junceiformis*. The Mediterranean hexaploid Sand Couch is to be known as *E. farctus*. The cytological and morphological distinctions between *E. farctus* and *E. junceiformis* were detailed by Simonet (1935a,b).

Figure 1. Inflorescences. A, *Elymus repens* f. *repens* lateral view, photo Richard Stace; B. *Elymus repens* f. *repens* abaxial view, photo Richard Stace; C. *E. repens* f. *aristatus*; D. *E. caninus*; E, *Hordeum secalinum*.

Nothogenera involving hybrids between *Hordeum* and couchgrasses

Hybrids between *Hordeum* and *Elymus* fall under the nothogeneric name ×*Elyhordeum* Mansf. ex Tsitsin & K.A. Petrova (1955). There is a later synonym ×*Elymordeum* Lepage (1957) (see under Taxonomic Conclusions). In addition there is a range of other nothogeneric names available to cater for treatments in which one or more of our four couchgrasses are placed in genera other than *Elymus*, and one catering for a split of *Hordeum*:

Triticum × *Hordeum* = ×*Tritordeum* Asch. & Graebn., *Syn. Mitteleur. Fl.* 2, Abth. 1: 748 (1902)
Agropyron × *Hordeum* = ×*Agrohordeum* E.G. Camus ex A. Camus, *Bull. Mus. Natl. Hist. Nat*. 33: 537 (1927)
Synonyms: ×*Agropyrohordeum* E.G. Camus ex A. Camus, *Rivièra Sci*. 21: 44 (1934)
×*Agropyrhordeum* P. Fourn., *Quatre Fl. France* 91 (1935)
×*Hordeopyron* Simonet, *Compt. Rend. Hebd. Séances Acad. Sci*. 201: 1212 (1935a)
Elytrigia Desv. × *Hordeum* = ×*Elytrordeum* Hyl., *Bot. Not.* 1953: 357 (1953)
Roegneria K. Koch x *Hordeum* = ×*Horderoegneria* Tzvelev, *Fl. Arct. URSS* 2: 241 (1964)
Sitanion Raf. ×*Hordeum* = ×*Sitordeum* Bowden, *Canad. J. Bot.* 45: 722 (1967)
Elymus × *Critesion* Raf. = ×*Elytesion* Barkworth & D.R. Dewey, *Amer. J. Bot.* 72: 772 (1985)

The genus *Hordelymus* (Jess.) Jess. ex Harz, *Landw. Samenk.* 2: 1147 (1885) should also be mentioned. This is not a nothogenus but a genus containing sexual non-hybrid species. However, the same name was coined as a nothogenus, ×*Hordelymus* Bachteev & Darevsk., *Bot. Zhurn. (Moscow & Leningrad)* 35: 191 (1950). This predates x *Elyhordeum* but is illegitimate (later homonym).

Another genus to note is *Rouxia* Husn., *Gram. Fr. Belg.* 76 (1899), which was created to accommodate *Agropyron rouxii* Gren. & Duval-Jouve as *R. hordeoides* Husn. This is not a nothogenus, but it was later wrongly treated as one to replace ×*Agrohordeum* by Kerguélen (1975), who made the combination ×*Rouxia rouxii* (Gren. & Duval-Jouve) Kerguélen.

As stated above, for present purposes we are placing all our four couchgrasses under *Elymus*, so all intergeneric hybrids with *Hordeum* come under ×*Elyhordeum*.

Discovery and distribution of ×*Elyhordeum* taxa in western Europe

Two hybrids have been reported previously and a further one has recently been discovered in North Lincolnshire, all involving *Hordeum secalinum* Schreb. (Fig. 1E) as one parent.

E. repens × H. secalinum *=* ×Elyhordeum langei *(K. Richt.) Melderis*

×*Elyhordeum langei* was discovered in July 1865 by Nielsen (1872) at Stubberup on the island of Sjælland (Zealand) in Denmark (Fig. 2), where it was known from 1865 to 1877. It was first recorded by Nielsen as *Agropyron repens* var. *hordeacea*, but his brief note did mention the possibility of its being a hybrid with *Hordeum secalinum*. This parentage was formally adopted by Lange (1886), but he did not give it a binomial. Nevertheless Richter (1890) described it as *Agropyron* × *langei*, and it was later authors who transferred it to nothogeneric status. The morphology of this hybrid was described in some detail by Vestergren (1925), without commenting on its nomenclature. Hansen (1960) mentioned it briefly in a survey of Danish *Elytrigia* hybrids, but shortly after (Hansen, 1965) reported a second site at Marstal on the small island of Ærøs, about 70 km from the first locality. There are records from there between 1961 and 1964, but it might have persisted longer. Hansen (d. 2008) told Henry Nielsen that the Ærøs population died out "about 30 years ago"; some was transplanted to the Copenhagen Botanic Garden (Hansen, 1965) but this also died out (Jens Christian Schou, pers. Comm., 2020).

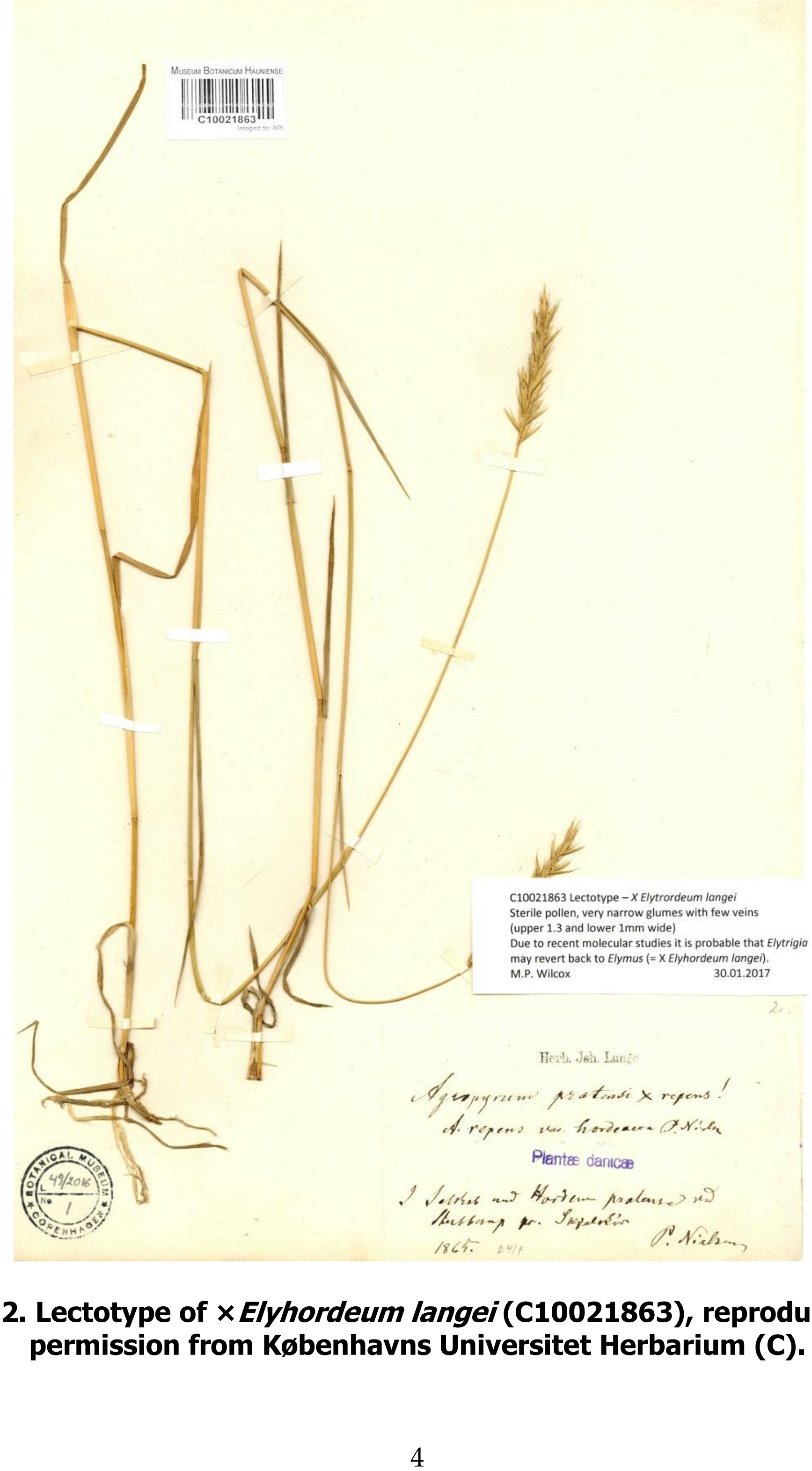

Figure 2. Lectotype of ×*Elyhordeum langei* (C10021863), reproduced with permission from Københavns Universitet Herbarium (C).

×*Elyhordeum langei* was discovered in the British Isles in 1945 by Mrs C.I. Sandwith (Hubbard & Sandwith, 1955a, 1955b) at Shirehampton, West Gloucestershire, where it was known until 1954, but there are no records since then, despite searches. In the past three decades other localities for this hybrid have been reported from West Gloucestershire, mostly by C. & M.A.R. Kitchen:

(a) in an semi-continuous zone along a *c*. 2 km stretch of the east bank of the lower reaches of the R. Severn south of Aust, at Northwick Warth, between Old Passage and just north-east of New Passage, ST552870 to ST563888 (first record made in 1991 by S. O'Donnell) (Fig. 3A).

Figure 3. Natural stands of ×*Elyhordeum langei*. A. Aust, W. Gloucs, with (left to right) Olga Krylova, Clive Lovatt and Clare Kitchen (Mark Kitchen); B. Tewkesbury, E. Gloucs (Clive Lovatt).

(b) south of Lydney, in at least three separate stands on the west bank of the R. Severn about 14 km north of the other stands, from Lydney Harbour to Aylburton Warth, ST62189999 to SO64430123 (first record made in 1993 by C. & M.A.R. Kitchen).

The hybrid has been confirmed in both areas by C. & M.A.R. Kitchen and C.M. Lovatt in 2020.

Elsewhere, confirmed records, which are all still extant, have been made by I.P. Green in 1990 at Alstone, North Somerset, ST310473; by S. Reynolds in 1990 at Ringmoylan, Co. Limerick, R405577; and by C.R. Pope & E.J. Clement in 2015 at Sandown Levels, Isle of Wight, SZ608852. All the above records of ×*E. langei*, in all four vice-counties, are maritime or estuarine, with definite or possible saline influence at least in the past. However, in July 2020 C.M. Lovatt discovered a fifth locality: two sizeable patches of the hybrid in rough grassland in Tewkesbury Nature Reserve close to the centre of Tewkesbury, East Gloucestershire, SO898323, which is very far from any salinity (Fig. 3B).

There are many other records of this hybrid from Britain, from localities as far north as Orkney, and many of them inland, but we cannot confirm that any of them are correctly identified. Those that we have seen (from W Cornwall, Berkshire, Bedfordshire, E. & W. Norfolk, Mid-W. Yorkshire, S. Northumberland and Orkney) are long-awned variants of *E. repens*, some of which Hubbard (1975) called 'nothomorph B' (see below).

Cauderon & Saigne (1961) and Cauderon (1962) stated that hybrids of this parentage had been synthesised at the Station d'Amélioration des Plantes, Clermont Ferrand.

Agropyron rouxii *Gren. & Duval-Jouve*

Grenier & Duval-Jouve (1860) described *A. rouxii* from material collected in 1858 (Roux & Blaise 67, **MPU**, **P**, Fig. 4) in saline meadows at Berre, near Marseille, Bouches-du-Rhône, France. Duval-Jouve (1870, pp. 76-77) reported that Roux had told him that the site had been soon destroyed by the construction of a soda factory, but that in 1869 H. Armand (Frère Lioberus) found the plant in the marshlands south-west of Roquehaute, on the coast of Portiragnes, Hérault, from which Duval-Jouve was given material. There are specimens from there in **MPU** collected up to 1877. Husnot (1899, pp. 395-396) also mentioned this locality as well as another in the same département "derrière la cabane Gilles à Mauguio". There are several sheets in **MANCH**, **MPU** and **P** from the latter locality dated from 1897 to 1901, all collected by E. Mandon (e.g. Dörfler's Herbarium Normale no. 3665, coll. 1898). On one sheet (the earliest, 09.06.1897) Mandon wrote triumphantly "Voila la meilleure trouvaille pour un graministe. Vive le Triticum rouxii". Although it was said to be abundant in the former place, it has unsuccessfully been sought there in recent years, the area having been developed for horse-riding (J.-M. Tison, pers. comm., 2019); it might persist in the latter locality. Camus (1958) also cited Giens, département Var.

Earlier specimens almost certainly of the same identity also exist (**P**, **K**), collected by P. Salzmann in 1819 and 1822. They are variously labelled " trouvé une seule fois près des salines de Villeneuve" [Villeneuve-lès-Maguelone, Hérault], and "Environs de Montpellier [Hérault], aux bords des étangs". The latter place possibly refers to Mauguio (J.-M. Tison, pers. comm., 2020). They were labelled by Salzmann as *Triticum salinum* Salzm., a herbarium name adopted later by Steudel (1841) but still a nomen nudum.

Figure 4. Holotype of ×*Elyhordeum rouxii* (MPU449820), reproduced with permission from Université de Montpellier Herbarium, Montpellier (MPU).

There are two 20th century reports. Cugnac & Simonet (1953) stated that "one of us" collected it in 1935 near Montpellier and near Palavas (Hérault), where it was very rare in the damp littoral meadows. It was later collected by D. Allen in the 1980s around the Étang de Vendres, extreme west Hérault, where it has since been unsuccessfully sought by the finder (J.-M. Tison, pers. comm., 2020). At present it is unknown in France, but there is a good chance that it persists somewhere in Bouches du Rhône or Hérault.

This plant has a disputed parentage. Duval-Jouve (1870) transferred the specific epithet to *Triticum* but did not mention hybridity. Later he (Duval-Jouve, 1875) seemed to favour a hybrid origin on the basis of its constant sterility, but would not commit himself before further studies, although Husnot (1899) claimed that Duval-Jouve believed it was "*Agropyrum elongatum* x *Hordeum maritimum*" [= *Elymus elongatus* x either *Hordeum secalinum* or *H. marinum*]. Camus (1934) similarly suggested that it was "*Agropyrum scirpeum* [= *Elymus elongatus*] x *Hordeum maritimum*", but only two years later (Camus, 1936) she opted for *E. athericus* x *H. secalinum*, which has been the consensus opinion ever since (Camus, 1958; Cugnac & Simonet, 1953; Tison & de Foucault, 2014). Husnot (1899) had earlier stated that E. Mandon, who had collected the plant from the wild in 1897 and later (see above), favoured the latter interpretation ("*Agropyrum littorale* x *Hordeum secalinum*").

The Lincolnshire plants

A single clump of a hybrid resembling ×*E. langei* was discovered near Lincoln in North Lincolnshire on 05.06.2014 by P. Kirby on a wide grassy road verge about 0.8 km outside the village of Kexby, SK886885. Since its discovery, P. Kirby has located the grass in two other locations: Thorpe Bridge (Sturton by Stow), SK9080, 13.09.2014, two small clumps on a grassy verge; and at Saxilby, SK8877, 26.06.2017, where one good clump grows on each side of a narrow track between fields (Fig. 5A). Material from Kexby was sent to one of us (MPW), who later visited the sites. These were tentatively identified as the hybrid *Elymus caninus* x *Hordeum secalinum* by MPW due to the densely tufted habit, the long curving inflorescences and the very long awns (Fig. 5B). Both putative parents occur at the sites. Since this was a new combination it initiated this study of the intergeneric hybrids between *Elymus* and *Hordeum*.

Cugnac & Simonet (1953) reported on their experimental crosses involving these two species, which were carried out in 1948 and 1950 with material from the Ardennes and employed *H. secalinum* as the female parent. They obtained 12 viable caryopsides from 100 pollinations. The F_1 plants were vigorous, floriferous, completely sterile, and morphologically resembled ×*E. rouxii*. The spikelets were mostly borne singly at each node, but some spikes had paired spikelets at some nodes.

Chromosome Counts

British material of *Elymus repens* and *E. athericus* is hexaploid (2n = 42); *E. junceiformis*, *E. caninus* and *Hordeum secalinum* are tetraploid (2n = 28) (Stace, 2019). The Mediterranean *E. farctus* is hexaploid (Simonet, 1935a, b).

The natural hybrid ×*E. langei* from Marstal on the island of Ærøs, Denmark, was counted soon after it was found there by Hansen in 1960 (Hansen, 1965).

Hansen sent material to Mme Y. Cauderon in Clermont Ferrand and to H. Christiansen at the Landbohøjskolen Arvelighedslaboratorium (Agricultural University), Denmark, and both counted it as 2n = 49. The obvious explanation of this is that the *Hordeum* parent contributed unreduced gametes.

Figure 5. Stand of ×*Elyhordeum kirbyi* at Saxilby, N Lincs. A. general view with its finder, Paul Kirby; B. closer view showing inflorescences.

Cauderon (1962) quoted 2n = 49 for *E. repens* x *H. secalinum*, but did not state the provenance of the specimens; the fact that she said that Simonet had studied it suggests confusion with ×*E. rouxii*.

Wild material of the French ×*E. rouxii*, collected in 1935, was counted by Simonet (1953), also as 2n = 49. This count also must have resulted from an unreduced gamete from *H. secalinum* and a reduced gamete from a hexaploid *Elymus*.

Synthesised hybrids of the parentage *E. repens* × *H. secalinum* (Cauderon & Saigne, 1961; Cauderon, 1962) had the expected 2n = 35. In 25 cells analysed there were means (and ranges) of 12.52 (8-16) bivalents, 8.28 (3-15) univalents, 0.4 (0-2) trivalents and 0.12 (0-1) quadrivalents. The authors explained this by suggesting that there was both *Elymus-Hordeum* and *Elymus-Elymus* pairing.

Synthesised *E. caninus* × *H. secalinum* had the expected tetraploid chromosome number (2n = 28), but apparently meiosis was not studied (Cugnac & Simonet, 1953).

Materials and Methods

Live material of the putative parental species, of ×*E. langei* from all extant British and Irish sites (except Lydney) and of the putative *E. caninus* × *H. secalinum* from all three sites in Lincolnshire, was collected (See Appendix 1 for list of accessions). Root tips were used for chromosome counts. Leaf material was dried with silica gel. Herbarium material of ×*E. langei* and ×*E. rouxii* was studied, via loans or scans from **C, K, MANCH, MPU** and **P**, using a dissecting microscope for inflorescence, sheath and leaf characters. Pollen was stained with aceto-carmine to estimate viability.

Chromosome Counts

Root tips were collected from potted plants, pre-treated overnight at 4°C in 2mM 8-hydroxyquinoline (BDH Chemicals), and then fixed in 3:1 (v/v) ethanol : acetic acid for 24 hr at 4°C (Bailey & Stace, 1992). Next, root tips were digested in 10mM citrate buffer containing 25U/ml pectinase from *Aspergillus niger* (Sigma-Aldrich), 20U/ml cellulase from *A. niger* (Sigma-Aldrich) and 20 U/ml cellulase 'Onozuka R-10' from *Trichoderma viride* (Duchefa Biochemie). The digested roots were then dissected and squashed in 45% (v/v) acetic acid (Schwarzacher et al., 1989). Chromosome preparations were preserved by quick-freezing on dry ice (Conger & Fairchild, 1953), and mounted in VECTASHIELD® Mounting Medium with DAPI, before being observed and imaged on a Nikon Eclipse Ci fluorescence microscope.

Molecular Analysis

Total genomic DNA (gDNA) was isolated from dried leaf material (20 mg) using DNeasy Plant Mini Kits (QIAGEN). The chloroplast gene *maturase K* (*matK*) was partially amplified from gDNA with angiosperm-specific primers AF and 8R (Ooi et al., 1995), purified and sequenced. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) was amplified from gDNA with plant-specific primers 17SE and 26SE (Sun et al., 1994), purified and sequenced. Putative hybrids were also sequenced from clones. Cloning was conducted using the QIAGEN PCR Cloning Kit; amplicons were ligated into pDrive and transformed into *E. coli* (DH5α). Recombinant plasmids were selected for by blue-white screening and the size of the insert determined by colony PCR with M13 primers. Plasmid DNA was isolated from cell cultures using the E.Z.N.A.® Plasmid Mini Kit (Omega Bio-tek). A minimum of six colonies was sequenced per accession. Sanger sequencing reactions were outsourced to Eurofins. Generated sequence reads were viewed, trimmed and blasted with Geneious R7 (created by Biomatters; available from http://www.geneious.com/). Additional sequences were downloaded from the

GenBank database (Supplementary information 1). Sequences were aligned using the Clustal W algorithm, and adjusted by eye. Copies acquired from the putative hybrid specimens were investigated by direct sequence comparison with putative parental taxa and by phenetic analysis. An UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering analysis was conducted on sequence data using Geneious Tree Builder. Genetic distance model = Jukes-Cantor. Bootstrapping = 10,000 replicates. Topology support threshold = 75%.

Results

Characters of ×Elyhordeum *and its parent genera*

The two parent genera, both with a simple spike-like inflorescence, have starkly different spikelet arrangements. The following characters refer only to those species involved in ×*Elyhordeum* hybrids.

In *Elymus* there is one spikelet per node, which is flattened broadside to the inflorescence axis (Figs. 1A & B, 6A & B), two basal glumes which are lateral to the spikelet, and (2)4-7(9) florets all of which, except the apical one or two, are fully bisexual. When one views the spikelet abaxially between the two glumes, one sees the lemmata of the florets alternating up the rhachilla (Figs. 1B & C, 7C). Awns on glumes and lemmata in *Elymus* vary from absent up to 15 mm in *E. repens* and 22 mm in *E. caninus*. At fruiting the spikelets break up between the florets, though often tardily so, and sometimes the rhachis also becomes somewhat fragile.

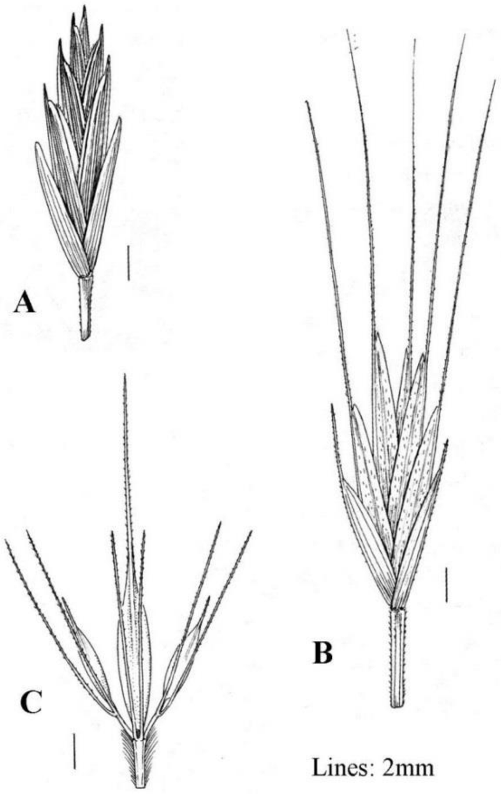

Figure 6. Spikelets of A. *Elymus repens*; B. *E. caninus*; C. *Hordeum secalinum*, reproduced from Cope & Gray (2009) with permission from Botanical Society of Britain and Ireland.

In *Hordeum* there are three spikelets per node, borne side by side (Fig. 6C). The central one has two glumes side by side in a more or less abaxial (not lateral) position, each very narrow and more or less reduced to a long awn. In each central spikelet there is a single floret which is orientated with the back of the lemma in an abaxial position, so that when the spikelet is viewed abaxially one sees the back of the lemma between the two glumes. The two lateral spikelets are similar but much narrower, because the floret lacks an ovary and sometimes anthers as well. The lemmata are always long-awned, in *H. secalinum* up to 14mm. At fruiting the inflorescence axis breaks up between the nodes, so the dispersal unit is a triplet of spikelets, with only one caryopsis.

×*Elyhordeum* combines features of both parents. Usually there is only one spikelet per node (rare exceptions, q.v.). This has two glumes, which vary in position from approaching lateral (as in *Elymus*) to more or less side-by-side and abaxial (as in *Hordeum*), and 1-2(3) florets. Within each spikelet the florets, if more than 1, alternate on each side of the rhachilla, as in *Elymus*, but the group is variably twisted up to 90° to those of *Elymus*. Where the twisting is at or close to 90° the back of the first lemma is abaxial and is what one sees between the two glumes (Figs. 7A, B, D & E). The twisting from a lateral-lateral position to an abaxial-adaxial plane often varies at different nodes in one inflorescence, but is rarely less than 45° and frequently is at a full 90°. The first (or only) floret is bisexual, the other(s) much reduced; the second is usually male and the third often lacks both anthers and palea. Very rarely a fourth greatly reduced floret exists. The lemmata are always awned but to varying degrees depending on parentage. At maturity the inflorescence axis can break up between the nodes, and sometimes the spikelet breaks up between the florets, but often tardily and sometimes scarcely at all.

*Characters and variation of ×*E. langei (E. repens ×H. secalinum)

Vestergren (1925) described the characters of this hybrid in detail, with good illustrations. It is a rhizomatous plant, a feature derived from *E. repens*, and can be very similar in habit and general appearance to *E. repens*, sometimes being difficult to pick out among a population of *E. repens*. Rhizomatous spread can be very extensive, and evidently allows for vegetative dispersal, especially in coastal habitats where wind and water have a marked eroding effect. By the R. Severn in West Gloucestershire there are two very large expanses each spreading over about 2 km, with varying degrees of continuity. In East Gloucestershire there are two patches several metres across, about 50 m apart. The original Shirehampton patch occupied only "about a square yard" (Hubbard & Sandwith, 1955b), but in Co. Limerick there is a patch *c.* 3 m across with one or two small satellite stands, and in Somerset and Wight it is scattered over a few metres.

In almost all cases there is only one spikelet per node. However, Vestergren (1925) and Hubbard & Sandwith (1955a) stated that sometimes a few lower nodes possess 2 or even 3 spikelets. Vestergren illustrated such a node (Fig. 7F), with one smaller spikelet beside the main one, suggesting it was male or sterile. Whether Hubbard found inflorescences like this in his Shirehampton material we do not know, but we saw no such plants in any British material until C.M. Lovatt, after making a close study of his Tewkesbury material, discovered a few instances and sent material to us for dissection. Nodes 2-4 from the inflorescence base were those seen affected. One node had two nearly equal spikelets side-by-side, each with 2 florets, but usually one of them had only one glume (Fig. 7G). In other cases the second spikelet was distinctly smaller. Evidently there is variation in this character, but a single spikelet at each node is by far the commonest situation.

Figure 7. Abaxial views of spikelets. A. ×*Elyhordeum langei*; B. ×*E. kirbyi*; C. *Elymus caninus*; D. ×*Elyhordeum langei*, from Vestergren (1925); E. ×*E. kirbyi*, drawn by Catherine Gregory; F. ×*E. langei*, bearing two spikelets at one node, from Vestergren (1925); G. ×*E. langei*, bearing two spikelets at one node, specimen from Tewkesbury, E Gloucs.

Although there is considerable variation in ×*E. langei*, it can be distinguished from *E. repens* by its spikelets with nearly always only one ovary and anther-containing floret (rarely two), and the twisting of its spikelet axis towards the abaxial position (to varying degrees), and from *H. secalinum* by having nearly always only one spikelet per node, usually more than one floret per spikelet and the much wider (not awn-like) glumes. Like *E. repens*, the foliage can be green to somewhat glaucous: field comments we have received vary from "not at all glaucous" to "slightly glaucous" or "somewhat glaucous". The indumentum of the leaves and presence or absence of auricles is variable between the two parents and is of little diagnostic value. There is variation in the sizes of the glumes and lemmata and the length of the awns. Where the glumes, lemmata and (especially) the awns are long, and therefore overlapping the spikelet(s) at the next node, the inflorescence appears relatively 'loose' or 'shaggy' (Fig. 8A), and does not resemble that of *E. repens*. Those plants with short glumes, lemmata and awns present a more compact, neater appearance (Fig. 8B) and more closely resemble *E. repens* (but are always distinguishable from it as above). The culms show similar vigour to those of *E. repens* (often *c.* 1 m tall, rarely up to 1.8 m), but are often less rigidly upright (Fig. 9) and are easily windblown. The inflorescences are *c.* 5-10 cm, varying from slightly curved to straight. The glumes are *c.* 1-1.5 mm wide, much wider than those of *H. secalinum*, but narrower than those of *E. repens* and with fewer (2-4) veins; they are 5-8 mm with very short awns rarely up to 6.5 mm. They are frequently asymmetrical, having one more vein on one side of the midrib than on the other side. The lemma of the first floret is mostly 7-8 mm, with an awn (1)3-5(10) mm. The anthers are 3-3.7 mm, indehiscent, and with the great majority (>90%) of the pollen grains shrunken and empty.

Figure 8. Inflorescences. A. ×*Elyhordeum langei* from Shirehampton, W. Gloucs, reproduced with permission from Royal Botanic Gardens, Kew; B. x *E. langei* from Tewkesbury, E. Gloucs, photo Richard Stace; C. ×*E. kirbyi*, from Kexby, N. Lincs, photo Richard Stace.

Hubbard (1975) concluded that there are two nothomorphs of ×*E. langei*, which he called A and B. Nothomorph A clearly shows the characters of both parents, and is the one described in the above paragraphs. To quote Hubbard (1975): "Nothomorph B is easily confused with *A. repens* var. *aristatum* Baumg. Among specimens referred to this variety are some with an articulated inflorescence-axis and with the internode remaining attached to the spikelet base. In these, the awned glumes are wider [than in nothomorph A] and 5-7-nerved, and the spikelets 3-5-flowered." "Nothomorph B, while usually male-sterile, has been found with perfect pollen and occasionally a well-developed caryopsis."

Figure 9. Culms of ×*Elyhordeum langei* at Sandown, Wight. Photo Colin Pope.

This concept was accepted uncritically by Cope & Gray (2009) and Stace et al. (2015). Study of specimens in **K** show how Hubbard changed his opinion over the years and was uncertain of the identity of some specimens. For example, a specimen that he collected in Berkshire in 1943 and identified as "*Agropyron repens* var." was later (1972) identified by him as the intergeneric hybrid, and annotated "rhachis disarticulating, good pollen!" Later many awned plants were identified as this hybrid from widely scattered localities in Britain, including some (in N. England and Scotland) well outside the range of *H. secalinum*. As stated above, we have examined such plants from at least eight vice-counties and can see no reason to identify any of them as the hybrid. Most show some degree of male or female fertility. A tardily disarticulating rhachis is often found in old inflorescences of *E. repens*, some spikelets of which can have as few as three (very rarely only 2) florets. We conclude that Hubbard's nothomorph B is referable to *E. repens* f. *aristatus* (Schumach.) Stace, in which the lemma awns can be up to 15 mm long (Fig. 1C). This conclusion is supported by the results of the molecular analysis (see below).

*Characters and identity of ×*E. rouxii

This taxon was illustrated and described in some detail by Duval-Jouve (1870, pl. XX, fig. 4) and Husnot (1899, pl. XXX). Its spikelet morphology, very like that of ×*E. langei*, clearly shows it belongs to the nothogenus ×*Elyhordeum*.

×*Elyhordeum rouxii* differs from ×*E. langei* mainly in its more glaucous leaf-blades ("vert glauque", Husnot, 1899; "un peu glauque", Duval-Jouve, 1870), which become strongly involute at an early stage, and its adaxial leaf surface having more pronounced ribs with a dense covering of very short (16-130 µm), slightly antrorsely directed, pointed hairs (prickle-hairs) (Fig. 10E), rather than low ribs without or with sparse hairs.

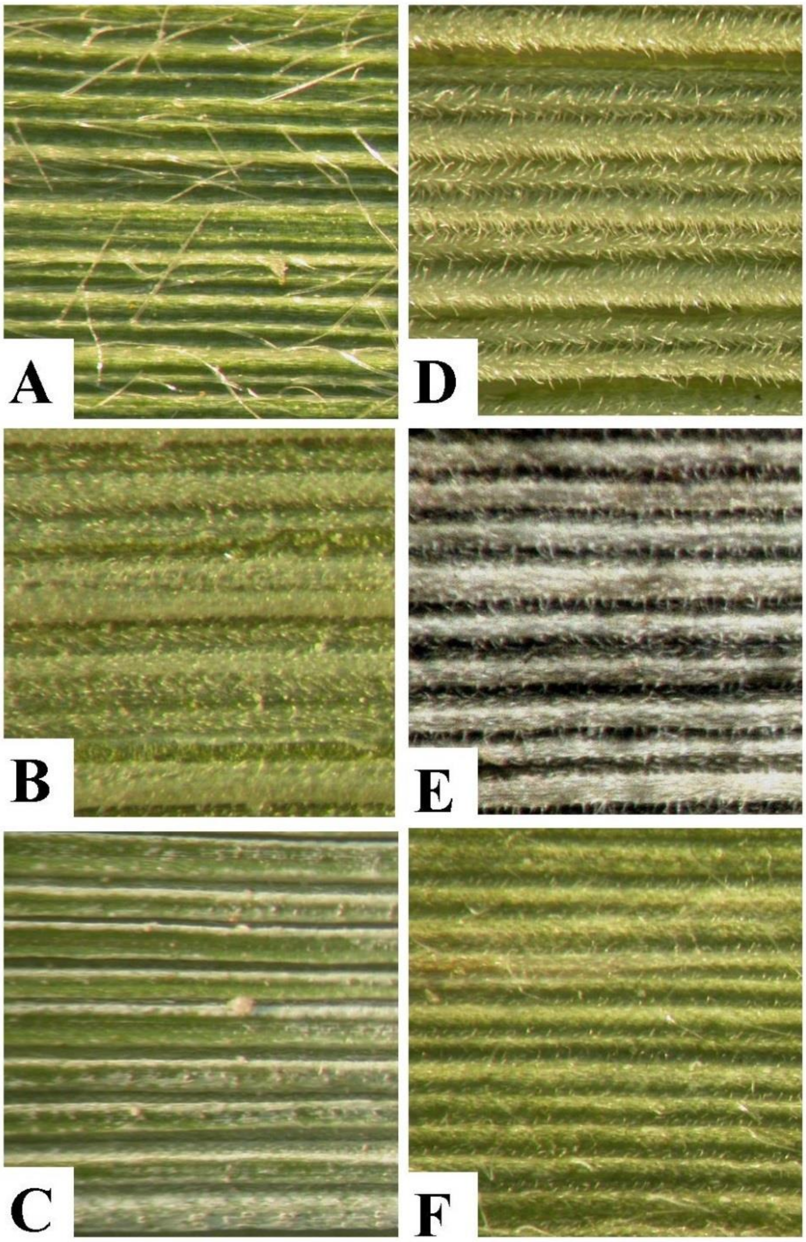

Figure 10. Adaxial leaf surfaces of A. *Elymus repens*; B. *E. elongatus*; C. *E. athericus*; D. *E. farctus*; E. ×*Elyhordeum rouxii*; F. *Hordeum secalinum.*

Duval-Jouve (1870) divided the perennial French *Triticum* into two groups: those with "rampant rhizomes" (most species); and those with a "fibrous stock, not at all rampant" (covering *T. caninum*, *T. elongatum* and *T. rouxii*). However, we believe that this might not be the case in ×*E. rouxii*. A good specimen of the latter in **K**, ex herb. Duval-Jouve, collected in June 1871 (i.e. after his paper was published) shows parts of well-developed rhizomes (Fig. 11). The locality given is Portiragnes;

the label is signed by Duval-Jouve, although it is not certain that he was the original collector. Either way, we have seen no evidence that Duval-Jouve had seen ×*E. rouxii* growing in the wild when he wrote his paper; if he had, it is likely he would have recognised whether it is rhizomatous or not. The other specimens that we have seen do not show rhizomes, but it is easy to pull out a tuft of a rhizomatous grass like *E. repens* or *E. athericus* without any trace of rhizome on the specimen.

Figure 11. Specimen of ×*Elyhordeum rouxii* ex herb. Duval-Jouve (K), collected from Portiragnes, Hérault, showing rhizomes.

Early suggestions that the *Hordeum* parent of ×*E. rouxii* is *'H. maritimum'* are not easily interpreted, because that name has been applied to both *H. secalinum* and *H. marinum*, but we believe it unlikely that the inbreeding annual diploid *H. marinum* was intended. With reference to the flora of the Rhône delta region, four species of *Elymus* are possible as the other parent. This is usually considered to be *E. athericus*, but *E. elongatus* was the first suggestion and *E. repens* and *E. farctus*, in terms of their distributions, are the other candidates.

The attribution of possibly diagnostic characters to the four candidate species is as follows:

Rhizomes: three species are strongly rhizomatous; *E. elongatus* is not at all so.

Leaf-blades: flat at first, but soon becoming involute in three species; in *E. repens* they frequently remain flat for some months, often into senescence.

Glaucousness: three species are glaucous; *E. repens* is usually non-glaucous but glaucous populations are not rare on the coast and occasionally inland.

Leaf adaxial ribs: three species have strong, pronounced ribs (at least as high as wide); *E. repens* has shallow ribs.

Leaf adaxial rib indumentum: *E. repens* is glabrous or with scattered long hairs (*c*. 1 mm); *E. athericus* has very sparse very short (<50 µm) prickle-hairs; *E. elongatus* has a dense to sparse covering of very short (16-50 µm) prickle-hairs; and *E. farctus* has a dense covering of short (80-190 µm) mostly spreading prickle-hairs.

Inflorescence: Congested, with spikelets overlapping on same side in *E. repens* and *E. athericus*; elongated, with spikelets not overlapping on same side in *E. elongatus* and *E. farctus*.
Chromosome number: Three species are hexaploids (2n = 42); *E. elongatus*, at least those plants counted in the western Mediterranean (subsp. *elongatus*), is diploid (2n = 14) (Cauderon, 1962).

Hordeum secalinum is not rhizomatous, has flat green (not glaucous) leaf-blades with shallow adaxial ribs with sparse to quite dense very short prickle-hairs and sometimes sparse longer hairs, has congested inflorescences and is tetraploid (2n = 28).

A species of *Elymus* forming a hybrid with *H. secalinum* (Fig. 10F) that has the features of ×*E. rouxii* might be expected to be rhizomatous, have involute glaucous leaf-blades with pronounced adaxial ribs bearing prickle-hairs >50 µm long, have a congested inflorescence, and (in view of ×*E. rouxii* having 2n = 49) be hexaploid.

Clearly, all four species have counter indications; in the case of *E. repens* (Fig. 10A) this involves four of the six characters. Of the other species, *E. athericus* has most often been suggested as a parent, but the dense leaf-rib prickle-hairs of ×*E. rouxii* (Fig. 10E) are not found in that species, which additionally usually has flat-topped ridges (Fig. 10C). Moreover, no specimens that we have examined have any indication of hairs on the sheath (free) margin (usually a diagnostic character of *E. athericus* and its hybrids, albeit usually with glabrous sheaths in the Mediterranean region, fide J.-M. Tison). Dense leaf-rib prickle-hairs are found in both *E. farctus* (Fig. 10D) and *E. elongatus* (Fig. 10B), but evidence against both of these species is the congested inflorescence of ×*E. rouxii*, and further against *E. elongatus* is its diploidy. The inflorescences of hybrids of *E. junceiformis* (elongated) with either *E. repens* or *E. athericus* (congested) on the Atlantic coast clearly show the influence of *E. junceiformis* in this character. The prickle-hairs of *E. farctus* are at least twice as long as those of *E. elongatus*; ×*E. rouxii* does have some hairs distinctly longer than those of *E. elongatus*. The presence/absence of rhizomes in ×*E. rouxii* remains a problem; Duval-Jouve (1870) stated that they were absent, but one of his later specimens clearly shows them.

Hence none of the species fully fits the criteria. We conclude that on present evidence *E. farctus* is the strongest contender, but that a more definitive statement of the parentage of ×*E. rouxii* must await the application of molecular methods to a plant rediscovered in the wild.

Characters and identity of Lincolnshire hybrids

These plants were originally thought to have a different parentage from ×*E. langei* because of their long awns (Figs. 7B & E), long arching inflorescences (Fig. 5B) and non-rhizomatous habit. Their general appearance resembles that of *E. caninus* (Fig. 1D), which occurs in all three localities, and this species was suspected as the *Elymus* parent. The 'loose' and 'shaggy' appearance of the inflorescences of some specimens of ×*E. langei* mentioned above is carried to a greater extreme in the Lincolnshire plants, in which the separate nodes often do not appear discrete without dissection (Fig. 8C). The inflorescences are usually longer than those of ×*E. langei*, 6-13(18) cm. In all the material we have examined there is a single spikelet at each node of the general construction described above, with 1-3 florets. The commonest situation is 2(-3) florets, the first bisexual, the second male, and the third (if present) very reduced and sterile. The floret axis is more often twisted through the full 90° so that the two glumes are usually clearly abaxial rather than lateral, showing the back of the first lemma between them (Figs. 7B & E). The glumes are 5-10 mm × *c*. 1 mm, with awns 5-8(10) mm and 3-4 veins. The first lemma is 7-10 mm with awns (8)10-18 mm. At anthesis the anthers and stigmas are often fully exserted, but this character seems to be variable, perhaps affected by the weather. In all cases the anthers (1.9-3.8 mm) are indehiscent and the pollen is >90% empty and shrunken, or in some cases very sparse, contrasting with the situation in the parents. The inflorescences break up only very tardily. It should be noted that in their artificial hybrids of this parentage Cugnac & Simonet (1953) recorded some paired spikelets.

There seems to be little variation between the plants at the three Lincolnshire sites.

Chromosome counts

The chromosome number of putative *E. caninus* × *H. secalinum* (material from Kexby, EK1) is 2n = 28, the same as in both putative parents. Similarly, ×*Elyhordeum langei* from Aust (EL4) has the expected 2n = 35 (parents 2n = 28 and 42). However, ×*E. langei* from Tewkesbury (EL5) has 2n = 49, indicating the contribution of an unreduced gamete from *H. secalinum*. This result mirrors the only two historical counts for ×*Elyhordeum*, 2n = 49 in both the French ×*E. rouxii* and the Danish ×*E. langei*.

Nuclear DNA marker

Generated ITS amplicons ranged in size from 853 to 856 bp; average GC content was 60.1%. The sexual non-hybrid species we sampled (*H. secalinum*, *E. athericus*, *E. caninus* & *E. repens*) gave clean sequence chromatograms for the full length of the ITS, which match previous submissions to the online GenBank database. *Elymus junceiformis* was not sampled in the present study, but an ITS1 sequence was acquired from GenBank (EU883122). The wild source of this material was not available, but it was reported as tetraploid and therefore is the Atlantic taxon, which is the one relevant to this study (Arterburn et al., 2011). In addition to awn-less individuals of *E. repens* (ER1, ER3), a long-awned variant was sampled (ER2), representing Hubbard's 'nothomorph B'. ITS sequences were consistent between all three individuals of *E. repens* sampled, and no signal of hybridisation was detected in the long-awned variant.

The ITS sequence chromatograms (5′ → 3′) of the putative *E. caninus* × *H. secalinum* (EK1, EK2, EK3) hybrids from Lincolnshire gave a clean signal for the first ~ 250 bp, with clear double peaks at sites where *Elymus* and *Hordeum* are polymorphic (Appendix 2). Within this region there is one nucleotide position (nt. 229) that differs between *E. repens* (T) and *E. caninus* (G), while *H. secalinum* is C. The putative *E. caninus* × *H. secalinum* (EK1, EK2, EK3) hybrids are G/C heterozygous at this site, indicating parental contributions from *E. caninus* and *H. secalinum*. *Elymus junceiformis* is also G at this nucleotide position, but can be ruled out as a parent by two earlier single nucleotide polymorphisms (SNPs; nt. 214, 223) that do not appear in the putative hybrids (Appendix 2). After the initial ~ 250 bp, the sequence trace becomes mixed and unintelligible, corresponding with a 4 bp indel (GGGT) between *E. repens/E. caninus* and *H. secalinum*, which appears to introduce a frameshift between two or more underlying sequences. Gene cloning was conducted on one of the putative *E. caninus* × *H. secalinum* hybrids (EK1) and two distinct ITS ribotypes were detected, designated copy 1 and copy 2. A direct sequence comparison of copy 1 matched *H. secalinum*, and a clustering analysis placed it in a strongly-supported *H. secalinum* group (100% bootstrap support (BS); Fig. 12). A direct sequence comparison of copy 2 matched *E. caninus*, and the clustering analysis placed it in a strongly-supported *E. caninus* group (87% BS). ITS sequence analysis therefore demonstrates parental contributions from both species and supports the identification of the Lincolnshire plants as the hybrid *Elymus caninus* × *Hordeum secalinum*.

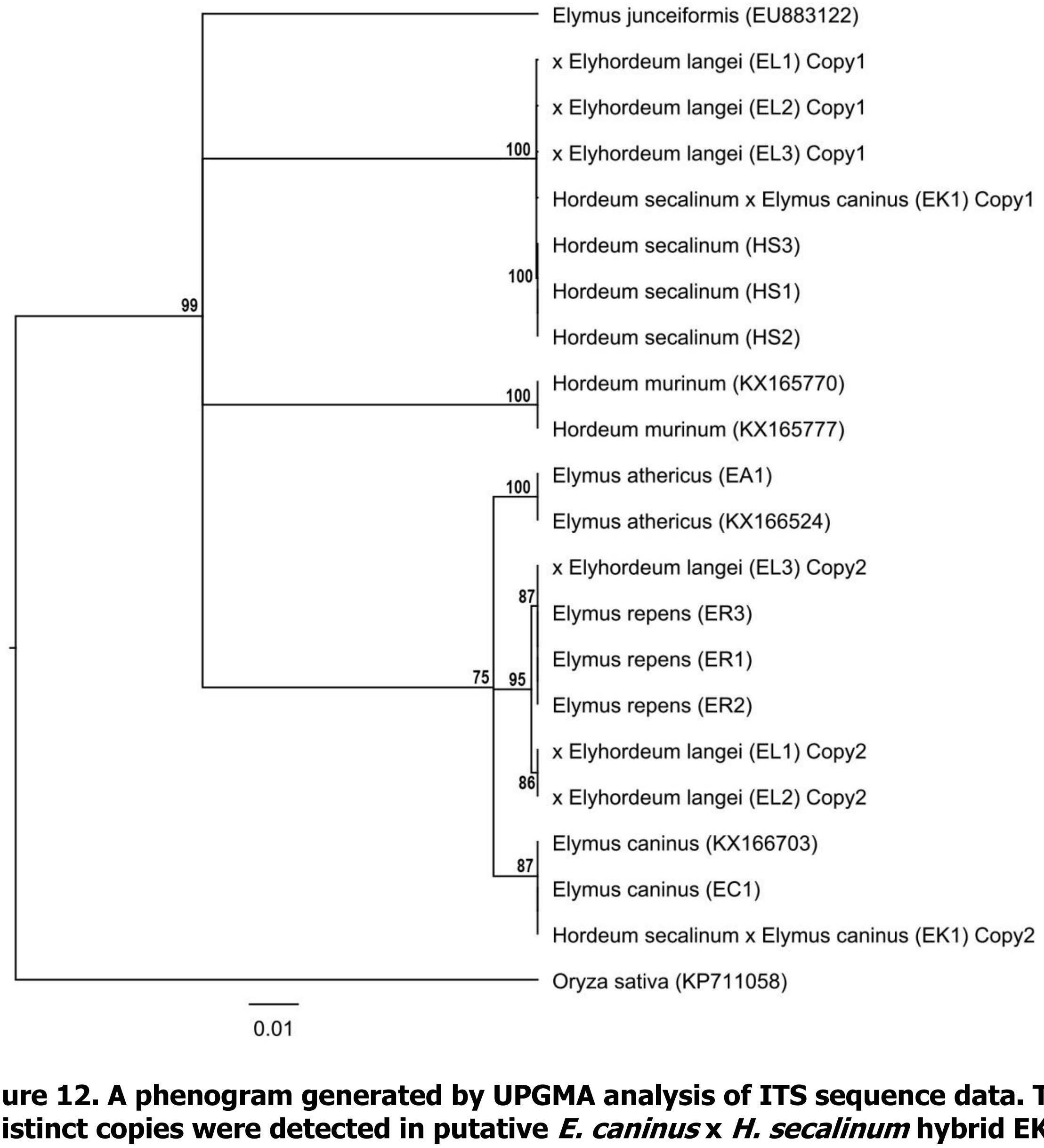

Figure 12. A phenogram generated by UPGMA analysis of ITS sequence data. Two distinct copies were detected in putative *E. caninus* x *H. secalinum* hybrid EK1 from Lincolnshire, copy 1 clustered with *H. secalinum* (100% BS) and copy 2 clustered with *E. caninus* (87% BS). BS values are displayed above nodes. Scale bar = 0.1 substitutions per nucleotide.

ITS sequences of ×*E. langei* were also analysed for comparison. As in the putative *E. caninus* x *H. secalinum* hybrids, ×*E. langei* individuals (EL1, EL2, EL3, EL4, EL5) gave clean sequencing signals (5′ → 3′) for the first ~ 250 bp with clear double peaks at sites where *Elymus* and *Hordeum* are polymorphic, but became mixed thereafter. At the informative site within the clean region (nt. 229) all five ×*E. langei* individuals sampled are T/C (Y) heterozygous, indicating parental contributions from *E. repens* (T) and *H. secalinum* (C). *Elymus athericus* is also T at this nucleotide position, but can be ruled out as a parent by three earlier SNPs (nt. 196, 209, 214) that do not appear in the hybrids (Appendix 2). Gene cloning was conducted on three individuals of ×*E. langei* (EK1, EK2, EK3) and two distinct ITS ribotypes were detected, designated copy 1 and copy 2. A direct sequence comparison of copy 1 copies matched *H. secalinum*, and the clustering analysis placed them in the strongly-supported *H. secalinum* group (100% BS; Fig. 12). A direct sequence comparison of copy 2 copies matched *E. repens* and the clustering analysis placed them in a strongly-supported *E. repens* group (95% BS). ITS sequence analysis demonstrates parental contributions from both species and confirms the identification of ×*E. langei* as the hybrid *Elymus repens* x *Hordeum secalinum*, which is clearly distinct from the Lincolnshire plants.

Chloroplast DNA marker

Generated *matK* amplicons were around 1.2 kb in length. *matK* chromatograms (5′ → 3′) gave a clean signal for the first 480 bp, but a significant proportion exhibited slippage downstream of a poly-$T_{(10)}$ region (nt. 471-481); all sequences were accordingly trimmed for comparison. Within this region all surveyed individuals of *Elymus* (EA1, ER1, ER2, ER3) are identical, with the exception of *E. caninus* (EC1), which possesses a single SNP (T → C, nt. 90). All three individuals of *H. secalinum* (HS1, HS2, HS3) are also identical within this region. However, *Hordeum* and *Elymus* possess distinct chloroplast haplotypes with eight intergeneric SNPs (nt. 2, 4, 90, 172, 184, 219, 359, 473).

The *E. caninus* x *H. secalinum* (EK1, EK2, EK3) hybrids all possess the *Hordeum* chloroplast haplotype. Direct sequence comparisons matched *H. secalinum* and a clustering analysis placed them in a strongly-supported *Hordeum* group (100% BS; Fig. 13). This indicates that *H. secalinum* is the maternal parent at all three sites of *E. caninus* x *H. secalinum* (EK1, EK2, EK3).

Four ×*E. langei* individuals (EL1, EL3, EL4 and EL5) also possess the *Hordeum* chloroplast haplotype. Direct sequence comparisons matched *H. secalinum* and the clustering analysis placed them in the strongly-supported *Hordeum* group (100% BS). However, one ×*E. langei* individual (EL2, from Limerick) possesses the *Elymus* haplotype. Direct sequence comparison matched *E. repens/E. athericus*, and the clustering analysis placed it in a strongly-supported *Elymus* group (99% BS)**.** This indicates that in four instances *H. secalinum* is the maternal parent of ×*E. langei*, but that the reciprocal cross is also possible as *E. repens* appears to be the maternal parent in the hybrid collected from County Limerick, Ireland.

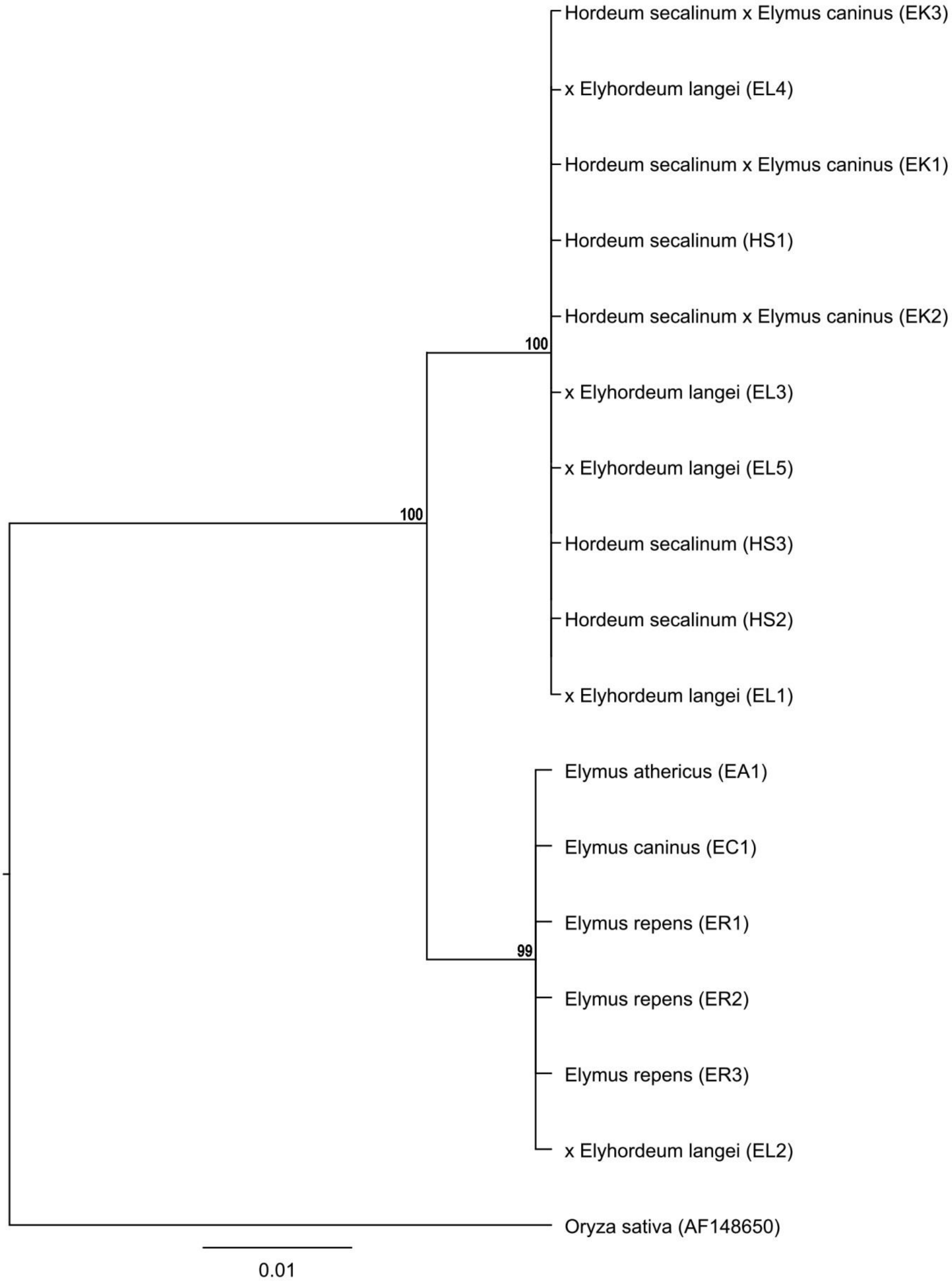

Figure 13. A phenogram generated by UPGMA analysis of partial *matK* sequence data. All ×*Elyhordeum* hybrids clustered with *H. secalinum* (100% BS), except EL2 which clustered with *Elymus* (99% BS). Scale bar = 0.1 substitutions per nucleotide.

Taxonomic Conclusions

×*Elyhordeum* Mansf. ex Tsitsin & K.A.Petrova, *Zuchter* 25: 164 (1955)
Synonym: ×*Elymordeum* Lepage, *Naturaliste Canad.* 84: 97 (1957)

×*E. langei* (K. Richt.) Melderis, *Watsonia*: 14: 394 (1983) = *Elymus repens* (L.) Gould x *Hordeum secalinum* Schreb.
Basionym: *Agropyron* x *langei* K.Richt., *Pl. Europ.* 1: 126 (1890)
Lectotype, selected here: Denmark: Sjælland: Stubberup, near Skælskør, "*Agropyron pratensi* x *repens*! *A. repens* var. *hordeacea*, P.Nielsen, 1865; Stubberup", **C** (C10021863) (Fig. 2)
Synonyms: *Agropyron repens* var. *hordeacea* Nielsen, *Bot. Tidsskr.* 5: 202 (1872)
×*Tritordeum langei* (K.Richt.) Asch. & Graebn., *Syn. Mitteleur. Fl.* 2, Abth. 1: 748 (1902)
×*Agrohordeum langei* (K.Richt.) E.G.Camus ex A.Camus, *Bull. Mus. Natl. Hist. Nat.* 33: 537 (1927)
×*Elytrordeum langei* (K.Richt.) Hyl., *Bot. Not.* 1953: 357 (1953)

×*E. rouxii* (Gren. & Duval-Jouve) Kerguélen, *Lejeunia* 110: 57 (1983) = *Elymus* sp. ×*Hordeum secalinum* Schreb.
Basionym: *Agropyron* × *rouxii* Gren. & Duval-Jouve, *Mém. Soc. Émul. Doubs*, sér. 3, 4: 391 (1860)
Holotype: Prairies salées de Berre près de Marseille, Bouches du Rhône, France, 11 juin 1858, H.Roux & Blaise 67, **MPU** (MPU449820, Fig. 4). Isotype: **P** (P00753805)
Synonyms: *Triticum* x *salinum* Salzm. ex Steud., *Nomencl. Bot.,* ed. 2, 2: 717 (1841), nomen nudum
Triticum × *rouxii* (Gren. & Duval-Jouve) Duval-Jouve, *Mém. Sect. Sci. Acad. Sci. Montpellier* 7(3): t. 20 (fig. 4) (1870)
Rouxia × *hordeoides* Husn., *Graminées* 4: 77 (1899)
×*Agropyrohordeum rouxii* (Gren. & Duval-Jouve) E.G.Camus ex A.Camus, *Rivièra Sci*. 21: 44 (1934)
×*Agropyrhordeum rouxii* (Gren. & Duval-Jouve) P.Fourn., *Quatre Fl. France* 91 (1935)
×*Hordeopyron rouxii* (Gren. & Duval-Jouve) Simonet, *Compt. Rend. Hebd. Séances Acad. Sci*. 201: 1212 (1935a)
×*Agrohordeum rouxii* (Gren. & Duval-Jouve) E.G.Camus ex A.Camus, *Ann. Soc. Linn. Lyon*, sér. 2, 79: 72 (1936)
×*Rouxia rouxii* (Gren. & Duval-Jouve) Kerguélen, *Lejeunia* 75: 297 (1975). Nothogeneric name incorrect

×*E. kirbyi* M.P.Wilcox, **nothosp. nov.** (*Elymus caninus* (L.) L. × *Hordeum secalinum* Schreb.)
Holotype: Wide, grassy road verge on Glentworth Rd, *c.* 0.8 km east of Kexby, North Lincolnshire, England, Grid reference SK886859, P. Kirby s.n., 05 June 2014, cultivated in garden of C.A. Stace, Suffolk, England, collected 16.07.2018 (**BM**) (Fig. 14)
Isotypes: **K, MANCH, LTR, P, C, MPU**.

Differs from ×*Elyhordeum langei* (*Elymus repens* × *Hordeum secalinum*) in its non-rhizomatous tufted habit, longer more curved or arching inflorescences (6-13(18) cm), glumes with longer awns (5-10 mm) and lemmata with much longer awns (8-18 mm).

Figure 14. Holotype of ×*Elyhordeum kirbyi*.

Discussion

Interspecific (and intergeneric) hybrids have been the subject of much study in the British Isles. The state of our knowledge in 1975 was summarised by Stace (1975), when the total number of hybrids in the wild with good confirmatory evidence was calculated at 624. This number included both hybrids that had arisen in the wild in the British Isles, and those that had been introduced as hybrids (e.g. *Crocosmia* x *crocosmiiflora*). In a later survey (Stace et al., 2015) the number had risen to 909, an increase of 45.7% in the intervening 40 years. Such surveys provoke active searching by our many field botanists, and since the last publication (up to September 2020) a further 23 hybrids have been reliably reported (and one deleted). To put these figures into the context of active hybridisation, 754 of the above 909, and 17 of the above 23, are hybrids that probably arose in the wild in the British Isles. This total of 771 spontaneous hybrids is remarkably high, and is certainly a fine illustration of the evolutionary potential of interspecific hybridisation. In addition, the recent splitting of *Rosa canina* into three, and of *Rosa caesia* into two, species (Bakker et al., 2019) has resulted (so far) in the recognition of 25 extra interspecific hybrids.

Several of the more recently discovered hybrids involved surprising combinations which would probably never have been predicted, e.g. *Berula erecta* x *Helosciadium nodiflorum (=* ×*Beruladium procurrens;* Desjardins et al., 2015) and *Elymus caninus* × *Hordeum secalinum* (= ×*Elyhordeum kirbyi*). There are now three combinations of ×*Elyhordeum* known from western Europe; more exist in North America and Asia and many have been produced experimentally. There can now be no doubt about the parentage of ×*E. langei* and ×*E. kirbyi*, but until ×*E. rouxii* is rediscovered in the wild its parentage will remain uncertain. As discussed above, two species of *Elymus* have been previously championed as putative parents for ×*E. rouxii*, but we consider that *E. farctus* is possibly the most likely. A noteworthy discovery from the molecular work is the parentage of ×*E. langei* from Aust, West Gloucestershire. Despite the occurrence of this hybrid close to vast swathes of *E. athericus*, with *E. repens* much less common in the vicinity, the latter species was the parent of the sample investigated.

It is remarkable that hitherto the only two chromosome counts of wild ×*Elyhordeum* hybrids have been 2n = 49, indicating the contribution of an unreduced gamete from *H. secalinum*. Moreover, one of three counts we report here (×*E. langei* from Tewkesbury) showed the same phenomenon. Unreduced gametes occur at an estimated frequency of <2% in natural plant populations (Ramsey & Schemske, 1998; Kreiner et al., 2017), but appear to be overrepresented in ×*Elyhordeum* hybrids. ×*E. langei* and ×*E. rouxii* are inter-ploidy crosses between tetraploid and hexaploid taxa, and the prevalence of unreduced gametes may be related to the direction of hybridisation. In the case of ×*E. langei*, our data indicate that tetraploid *H. secalinum* is more commonly the seed parent and hexaploid *E. repens* more commonly the pollen parent. This results in the potential for paternal-excess, where the father has a higher ploidy level than the mother. While not unprecedented, paternal-excess crosses are typically less successful than maternal-excess crosses at producing viable, odd-ploidy seed, e.g. triploids, pentaploids etc. (see Ramsey & Schemske, 1998 and references therein; see Vallejo-Marín et al., 2016 and references therein). A number of possible mechanisms have been suggested that modulate this phenomenon, including cytonuclear incompatibilities (Tiffin et al., 2001), ploidy imbalances in endosperm/embryo genome ratio (Burton & Husband, 2000), and/or ploidy imbalances maternal/paternal genome ratio (Haig & Westoby, 1989; Köhler et al., 2010). It is possible that unreduced gametes originating in the female line serve to compensate for this maternal/paternal disparity in ploidy, and restore an increased female contribution, thereby increasing the likelihood of viable hybrid seed. However, this is somewhat speculative and it should be noted that the female *H. secalinum* × male *E. repens* cross is viable and very vigorous without the contribution of unreduced gametes, as was shown in the hybrid from Aust.

With respect to the three wild populations of ×*E. langei* with a known chromosome number, the one from Tewkesbury differs in some morphological features from those from Denmark and Aust. In its relatively stiff erect stems, short awns and non-overlapping spikelets it appears closer to *E. repens* than to *H. secalinum*, which is the opposite to what might be expected in a hybrid carrying twice the genetic load of *H. secalinum*. However, the Danish plants, which also carried a double load from *H. secalinum*, appeared more intermediate.

The highly restricted distribution of the ×*Elyhordeum* hybrids is not easily explained. ×*E. rouxii* has been found only in the Rhône delta region of France, ×*E. kirbyi* is known from only one English county, and ×*E. langei* has been recorded in only two localities in Denmark, one in Ireland and five in England, despite the parents in each case being widespread and frequently growing close together throughout much of western Europe. Moreover, in the case of ×*E. langei*, all but one of the eight localities is estuarine or coastal. The two most obvious explanations are that these hybrids are much commoner than their records suggest, being overlooked by recorders, or that there are particular environmental factors which limit or favour successful hybridisation. There is some evidence for the latter because there are indications that within each of the areas several hybridisation events have taken place. For example, in the case of ×*E. kirbyi*, which is non-rhizomatous so has poor dispersal potential even with human disturbance, there are three separate locations spread over about 10 km, two of them with more than one clump. ×*E. rouxii* has a number of localities stretching over about 240 km, up to *c*. 2 km from the coast (J.-M. Tison, pers. comm., 2020). The distribution of ×*E. langei* in each of its sites is summarised above; undoubtedly extensive vegetative dispersal has taken place, but there is circumstantial evidence of multiple hybridisation events as well. It seems that the areas supporting hybrids are indeed 'hotspots', with as yet unexplained causes. Other gaps in our knowledge are the ploidy level of the plants at four of the six extant sites of ×*E. langei*, and whether the chromosome number is constant across the large populations in West and East Gloucestershire. In the case of ×*E. rouxii*, what is its parentage, and indeed was this the same at all sites?

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Supplementary Information

Accessions used in the current study and their associated metadata. Sequences generated in the current study are denoted with a double asterisk (**).

Taxon – location; accession code; ITS GenBank accession number(s); *matK* GenBank accession number(s).

Elymus athericus* (Link) Kerguélen** – a) Alstone, N Somerset, England; EA1; ITS MW142540** *matK* MW148213**. ***Elymus caninus* (L.) L.** – a) Thorpe-le-Fallows, N Lincolnshire, England; EC1; ITS MW142541** *matK* MW148214**. ***Elymus junceiformis* (Á. & D. Löve) Hand & Buttler** – a) unknown; n/a; ITS EU883122 *matK* n/a. ***Elymus repens* (L.) Gould** – a) Kexby, N Lincolnshire, England; ER1; ITS MW142542** *matK* MW148215** – b) Kexby, N Lincolnshire, England; ER2; ITS MW142543** *matK* MW148216 – c) Alstone, N Somerset, England; ER3; ITS MW142544** *matK* MW148217**. ***Hordeum murinum* L.** – a) Cardiff, Wales; n/a; ITS KX165770 *matK* n/a – b) Cardiff, Wales; n/a; ITS KX165777 *matK* n/a. ***Hordeum secalinum* Schreb.** – a) Kexby, N Lincolnshire, England; HS1; ITS MW142545** *matK* MW148218** – b) Alstone, N Somerset, England; HS2; ITS MW142546** *matK* MW148219** – c) Brading Marshes, Isle of Wight, England; HS3; ITS MW142547** *matK* MW148220**. *Oryza sativa* L. – a) China; n/a; ITS KP711058 *matK* n/a. **x *Elyhordeum kirbyi M.P. Wilcox – a) Kexby, N Lincolnshire, England; EK1; ITS MW142524**, Copy 1 MW142525**, Copy 2 MW142526** *matK* MW148205** – b) Thorpe-le-Fallows, N Lincolnshire, England; EK2; ITS MW142527** *matK* MW148206** c) Saxilby, N Lincolnshire, England; EK3; ITS MW142528** *matK* MW148207**. **x *Elyhordeum langei* (K. Richt.) Melderis** – a) Alstone, N Somerset, England; EL1; ITS MW142529** Copy 1 MW142530** Copy 2 MW142531** *matK* MW148208** – b) Ringmoylan, County Limerick, Ireland; EL2; ITS MW142532** Copy 1 MW142533** Copy 2 MW142534** *matK* MW148209** – c) Brading Marshes, Isle of Wight, England; EL3; ITS MW142535** Copy 1 MW142536** Copy 2 MW142537** *matK* MW148210** – d) Aust, W Gloucestershire, England; EL4; ITS MW142538** *matK* MW148211** – e) Tewkesbury, E Gloucestershire, England; EL5; ITS MW142539** *matK* MW148212**.

Appendix 1. Accessions collected and used in the current molecular study.

Taxon	Code	Location	Collector	Field notes
Hordeum secalinum	HS1	Kexby, N. Lincolnshire, England. SK886759	M.P. Wilcox	
	HS2	Alstone, N. Somerset, England. ST310447	M.P. Wilcox	
	HS3	Brading Marshes, Isle of Wight. SZ6081852.	E. Clement & C. Pope	
Elymus athericus	EA1	Alstone, N. Somerset, England. ST310635	M.P. Wilcox	"Viable pollen"
Elymus caninus	EC1	Thorpe-le-Fallows, N. Lincolnshire, England. SK903728	M.P. Wilcox	
Elymus repens	ER1	Kexby, N. Lincolnshire, England. SK886329	M.P. Wilcox	"Awn-less"
	ER2	Kexby, N. Lincolnshire, England. SK886209	M.P. Wilcox	"Long-awned"
	ER3	Alstone, N. Somerset, England. ST3104473	M.P. Wilcox	"Awn-less"
×*Elyhordeum langei*	EL1	Alstone, N. Somerset, England. ST310465	M.P. Wilcox	"Both parents present"
	EL2	Ringmoylan Quay, Pallaskenry, County Limerick, Ireland. R4050857	M.P. Wilcox	
	EL3	Brading Marshes, Isle of Wight. SZ608185	E. Clement & C. Pope	
	EL4	Aust, W. Gloucestershire, England. ST559881	C. & M. Kitchen	"Much E. athericus nearby"
	EL5	Tewkesbury, E. Gloucestershire, England. SO898323	C .Lovatt	
H. secalinum x *E. caninus*	EK1	Kexby, N. Lincolnshire, England. SK886009	M.P. Wilcox	
	EK2	Thorpe-le-Fallows, N. Lincolnshire, England. SK903728	M.P. Wilcox	
	EK3	Saxilby, N. Lincolnshire, England. SK881608	M.P. Wilcox	

Appendix 2. Species-specific SNPs of nrDNA ITS for the first 253 bp (5′ → 3′), the region upstream of a 4 bp indel (GGGT) between *E. repens, E. caninus* and *H. secalinum*. The putative *E. caninus* x *H. secalinum* hybrids from Lincolnshire are heterozygous at all sites polymorphic between *H. secalinum* and *E. caninus*, indicating additive parental contributions from both species.

Taxon	Accessions	nrDNA ITS Nucleotide Position															
		180	**183**	**196**	**197**	**203**	**204**	**206**	**209**	**211**	**212**	**214**	**215**	**221**	**223**	**228**	**229**
Hordeum secalinum	HS1, HS2, HS3	T	T	G	C	G	T	G	C	A	A	C	A	C	G	G	C
Elymus athericus	EA1	A	C	T	C	A	C	A G	T	G	T	T	G	C	G	T	T
Elymus caninus	EC1	A	C	G	T	A	C	G	C	G	T	C	G	C	G	T	G
Elymus junceiformis	--	A	C	G	T	A	C	G	C	G	T	T	G	C	A	T	G
Elymus repens	ER1, ER2, ER3	A	C	G	T	A	C	G	C	G	T	C	G	C T	G	G T	T
× *Elyhordeum langei*	EL1, EL2, EL3, EL4, EL5	T A	T C	G	C T	G A	T C	G	C	A G	A T	C	A G	C T	G	G T	C T
H. secalinum x *E. caninus*	EK1, EK2, EK3	T A	T C	G	C T	G A	T C	G	C	A G	A T	C	A G	C	G	G T	C G