



# The potential for endozoochorous dispersal of temperate fen plant species by free-roaming horses

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## Keywords

Konik; Landscape scale; Plant traits; Seedling emergence; Wetland restoration; Wicken Fen

## Nomenclature

Nomenclature follows Rodwell (1991, 1995) for plant communities and Stace (2010) for vascular plantspecies

## Abbreviations

NNR = National Nature Reserve.

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## Introduction

Large herbivores can contribute to species dispersal via the ingestion and subsequent defecation of plant propagules (endozoochory; Janzen 1984; Vellend et al. 2003). Germinable seed were observed to be retained in deer for more than 3 d (Vellend et al. 2003), cattle for 2 to 3 d (Simao Neto et al. 1987) and horses for up to 12 d (Janzen 1982). Consequently, and depending on the home range of the grazing animal, consumed seeds have the potential to be deposited in dung many kilometres from the parent plant (Pakeman 2001).

## Abstract

**Questions:** Can free-roaming Konik horses digest and subsequently disperse seeds of plant species growing in a temperate fen environment? Which species have the potential to be dispersed internally (endozoochory), and do they share common plant and seed traits? How could potential dispersal via endozoochory impact upon grazing management and the natural recolonization of wetland vegetation at a landscape scale?

**Location:** Wicken Fen NNR, Cambridge, UK.

**Methods:** Twelve dung samples were collected for one calendar year (Jun 2009–May 2010) from a free-roaming herd of Konik horses grazing vegetation at Wicken Fen NNR. Germinable seed content of the dung was determined by means of a seedling emergence study. Species presence and estimated cover/abundance were recorded for the grazed (background) vegetation in spring and summer 2009.

**Results:** Almost one-third of all species present in the background vegetation were also identified in the dung samples. There was an equal split between graminoids and forbs, but substantially more graminoid germinable seeds. Species were dispersed year-round, with the number of species peaking in Sep and again in Dec. Of the 13 traits measured, there were significant differences between dung and background vegetation for six traits. Mean values for seed release heights (minimum and maximum) and dispersule weight were significantly lower in species found in the dung, whilst dung species had significantly greater numbers of seeds per shoot, inferred Ellenberg nitrogen values and seed bank persistence.

**Conclusion:** Year-round free-roaming grazing has the potential to make a considerable contribution to the long-range dispersal of species growing in temperate fen habitats, and is likely to complement alternative dispersal strategies. However, germination and establishment of species post-dispersal fate will be governed by a wide range of environmental conditions.

Free-roaming wild herbivores would once have played an important role in the postglacial transportation of species across large distances (Heinken et al. 2002; Mouissie 2004; Jaroszewicz et al. 2009). In more recent times, traditional grazing practices involving the seasonal movement of domesticated livestock (e.g. transhumance, droving) would have facilitated the dispersal of species along defined migration routes (Duffey 1968; Bruun & Fritzboeger 2002). However, there have been substantial changes to or abandonment of traditional grazing systems over much of northwest Europe throughout the 20th century (Laiolo et al. 2004; Peco et al. 2005; Poschlod et al. 2005).

Changes in grazing practices have coincided with the widespread destruction of temperate fen ecosystems (Moore 1997; Jansen et al. 2000), so that fragments of remnant fen habitat are now isolated within a highly modified landscape. The loss of dispersal vectors and metacommunities (regional species pools connected by dispersal mechanisms; Leibold et al. 2004) reduces the probability of populations' colonizing or recolonizing an area (MacArthur & Wilson 1967; Hanski 1998) and therefore increases the probability of local extinctions (Harrison et al. 2000). The survival chances of species populations is therefore likely to be enhanced for those species that are able to disperse across long distances (Ozinga et al. 2004) and into suitable habitat. The ability to disperse over long distances is also likely to be advantageous in the face of future climate change. There is, therefore, a pressing need to: (1) re-establish connectivity between intact nature reserves or to substantially expand reserve boundaries through habitat restoration; and (2) quantify the long-distance (i.e. >100 m, following Cain et al. 2000) dispersal potential of plant species to better inform the strategies used in restoration.

In an attempt to repair ecological fragmentation, habitat restoration projects are increasingly taking place over a landscape scale (e.g. Wigbels 2000; Colston 2004). In southeast lowland UK, a number of such projects have been initiated in low-lying areas of fenland that were drained for agriculture in the 17th century. These projects aim to increase the area of wetland habitats and the levels of connectivity in the landscape. However, dispersal vectors such as hydrochory (e.g. natural flooding events), which would have been present in these low lying fen areas, are now almost absent in the landscape due to increased flood control, and many species classified as wind-dispersed (e.g. Kleyer et al. 2008) have been shown to rarely travel >100 m from the parent plant (Bullock & Clarke 2000; Jongejans & Telenius 2001; Tackenberg et al. 2003). Consequently, the contribution of large herbivorous mammals to the long-range dispersal of fen vegetation may be of considerable importance to the developing habitats in the new landscape-scale restoration projects.

Plant functional traits associated with successful internal dispersal of diaspores are hypothesized to be linked with edible seed casings, seed weight, seed shape and resistance to digestive processes (e.g. Janzen 1984; Pakeman et al. 2002; Couvreur et al. 2005). Numerous studies have examined the potential for endozoochorous seed dispersal across a range of dry grassland and heathland habitats (Welch 1985; Middleton & Mason 1992; Malo & Suárez 1995; Miller 1996; Eichberg et al. 2007; Kuiters & Huiskes 2010). However, there are very few studies concentrating on endozoochorous transport within wetland systems (Pakeman et al. 2002), and none within a temperate fen environment. In order to learn more about the potential

for livestock to carry out long-range dispersal of plants associated with such habitats, the main objectives of the study described here were to:

- 1 Determine the viable seed content of dung collected from free-roaming Konik horses grazing a species-rich UK fenland National Nature Reserve (NNR);
- 2 Examine temporal variation in dung species composition;
- 3 Investigate the similarities and differences between species transported by endozoochory and background vegetation traits;
- 4 Discuss the implications for grazing management and landscape-scale restoration.

## Methods

### Study site

Wicken Fen NNR, situated 25 km north of Cambridge (UK) (52°18' 24 N, 0°16' 51 E), is one of the last undrained remnants of what would once have been a vast interconnected landscape of floodplain mire in lowland East Anglia, some 3,850 km<sup>2</sup> in size (Moore 1997). The NNR is 159 ha in size and contains plant assemblages associated with low fertility floodplain fens on undrained alkaline peat (McCartney & de la Hera 2004), including fen meadow, sallow carr and tall herb fen. The study site is located within a 56.2-ha unfenced section of the reserve known as 'Verralls Fen', which is bounded on all sides by water-filled ditches. Following substantial scrub clearance operations from 2000 to 2001, the area has been extensively grazed year-round by a free-roaming herd of Polish Konik horses (henceforth referred to as Koniks, as the Polish word *konik* translates as 'horse' or 'pony'). Koniks were introduced because of similarities to the original Eurasian wild horse, including a resilient immune system, tolerance for harsh and wet conditions and an ability to graze coarse vegetation.

The Wicken Fen Vision is a landscape-scale restoration project bordering the NNR. The project is in the process of acquiring ca. 3000 ha of land to begin to address the problems of species isolation and dispersal. Restoration of vegetation within the project areas is based on an 'open-ended' approach (Hughes et al. 2011), which promotes natural colonization by flora and fauna and the repair (*sensu* Whisenant 1999) of ecosystem structure and biodiversity to establish ecosystem resilience (Breedlow et al. 1988), rather than seeking to replicate historic reference systems. The restoration areas are also grazed by Koniks but are not currently linked to the NNR. In anticipation that this link may one day be made, this study investigated the seeds in dung of the horses grazing Verralls Fen on the NNR to explore the potential for these horses to transfer species from the NNR to the restoration land.

### Dung sampling

Dung from the Konik herd (which numbered 13 animals in total) was collected on seven separate occasions (once every 3 wks) throughout the main plant fruiting season between the months of Jun and Oct 2009. On each visit, samples were collected from the first three Koniks to defecate. Five additional visits to collect horse dung were undertaken at regular intervals outside of the main fruiting season so that an analysis for a full calendar year could be undertaken. To avoid contamination by wind-blown seed, only dung that was observed as freshly deposited was collected. The lowermost layer was left behind to avoid contamination by seed lying on the soil surface. Fresh dung collected at each visit was homogenized and immediately placed in cold storage (constant 3 °C) for 4 wks to promote stratification. Samples were then spread thinly and fully dried in a sealed room at a constant 30 °C for 5–7 d. Once dried, a subsample of 1 kg (dry weight) representing a single site visit (hereafter referred to as a 'sample') was stored at –20 °C to maintain seed viability (following Linington 2003) until commencement of the seedling emergence experiment in Apr 2010.

### Germinable seed content

In Apr 2010, all samples ( $n = 12$ ) were transferred to a glasshouse located within Cambridge University Botanic Gardens. Before placing the material into seed trays, each sample was separated into four subsamples of 250 g (dry weight) and homogenized in a volume of distilled water (following Kuiters & Huiskes 2010). The resultant 'slurry' was then spread in a thin layer onto a mixture of sand and sterilized peat (1:1). Trays without dung samples were also present to detect germination from the compost and dispersal into the greenhouse. This method is similar to that devised by Ter Heerdt et al. (1996), but does not employ the use of fine sieves due to the high content of dead vegetative matter present in the dung. No preparation of dry dung after grinding was used as this may damage large seeds (Cosyns & Hoffmann 2005).

Seed trays ( $n = 48$ , excluding controls) were placed on capillary matting and regularly watered from below. Seedlings were identified at the earliest stage possible, recorded and then removed. Seedling emergence was recorded for 6 mo. Species that we were not able to identify at seedling stage were potted on and identified when diagnostic features became visible.

### Background vegetation

A comprehensive list of all species present in the vegetation (hereafter referred to as the background vegetation)

was recorded in spring and summer 2009. Cover/abundance for the entirety of the study area was estimated for each background species by undertaking a series of line-transect walks within the study area. Cover/abundance was estimated using the DOMIN scale [1 = <4% (few individuals); 2 = <4% (several individuals); 3 = <4% (many individuals); 4 = 4–10%; 5 = 11–25%; 6 = 26–33%; 7 = 34–50%; 8 = 51–75%; 9 = 76–90%; 10 = 91–100%]. However, the size of the grazed area (56 ha) within which horses were free to graze and the subsequent uncertainty surrounding where and when species had been consumed meant that a strict quantitative comparison of species occurring in the background vegetation and those germinating from the dung was not possible. Species presence/absence data were therefore used to compare functional traits of species that were found in the dung with traits of species that were in the background vegetation.

### Numerical analysis

Species functional traits were chosen to reflect both seed characteristics found to be important in endozoochory in the literature and plant characteristics that influence choice of species grazed by horses. Information on species functional traits was gathered from three principal sources. Inferred Ellenberg values for nitrogen (N), light (L) and moisture (F) were taken from PLANTATT (Hill et al. 2004). Dispersule weight, shape, and Competitor-Stress tolerator-Ruderal (C-S-R *sensu* Grime 1977) characteristics were taken from the Electronic Comparative Plant Ecology (Grime et al. 2007). Information on seed bank longevity, seed release heights and number of seed produced per shoot was assembled from the LEDA traitbase (Kleyer et al. 2008). Vegetation classification for dung species content was carried out using the program MAVIS (Modular Analysis of Vegetation Information Systems; Smart 2000).

Following the amalgamation of seed tray data for each sample, mean trait values for each dung sample ( $n = 12$ ) were calculated from presence of species in each sample and the trait score. Mean trait values for background vegetation were calculated as presence of all species that were recorded in the vegetation but that were not present as germinants in the dung samples, and the trait score. One-sample *t*-tests were performed to test for differences between dung and background vegetation traits. For each trait, the mean for each sample ( $n = 12$ ) was used to test against a hypothesized mean. For the purpose of this analysis, the background vegetation mean replaced zero as the hypothesized mean. Statistical analyses were carried out using Minitab v.14 (Minitab Inc., State College, PA, USA). Abundance data from the seedling emergence study were used to examine temporal patterns in dung species content.

## Results

The background vegetation in the grazed study area comprised 135 species recorded across a complex mosaic of four main vegetation types; *Peucedano-Phragmitetum australis* tall herb fen, *Cirsio dissecti-Molinietum* fen meadow, *Phragmitetum australis* reed bed and *Cladietum marisci* sedge beds (Table 1). Background species with an estimated cover/abundance of  $\geq 10\%$  across the 56-ha site included graminoids such as *Juncus subnodulosus*, *Calamagrostis epigejos*, *Calamagrostis canescens*, *Cladium mariscus*, *Phragmites australis*, *Phalaris arundinacea*, *Poa trivialis* and *Molinia caerulea*. Many graminoids and forbs that were recorded at the study site were locally abundant within areas of  $\leq 1$  ha, but did not comprise  $\geq 10\%$  cover/abundance when the study area was considered as an entire unit. Two forbs (*Thysetium palustre* and *Lathyrus palustris*) categorized by Cheffings et al. (2005) as 'vulnerable' and 'near threatened', respectively, in a UK context were recorded in low numbers within the background vegetation but were not recorded from the dung samples.

All species that germinated from the seedling emergence experiment were also found within the background vegetation. A total of 2548 seedlings of 41 species (30.4% of all background species) from 18 plant families were recorded from the dung samples (Table 1). Graminoids accounted for 48.8% of all species and 82.0% of all seedlings, whilst forbs made up 51.2% of species but only 18.0% of all seedlings. The mean number of germinable seeds for each collection date was  $212.3 \text{ kg}^{-1}$ , with the highest number of seeds  $\text{kg}^{-1}$  found in mid-Aug ( $720 \text{ kg}^{-1}$ ; Fig 1). The greatest number of species was found in the mid-Sep sample, and the lowest number in the mid-Jun sample (Fig. 2). The *Juncaceae* and *Poaceae* were the most dominant plant families in the dung samples, accounting for 34.14% of all species (12.2% and 22.0%, respectively), and 80.5% of all seedlings (64.8% and 15.7%, respectively). *Berula erecta*, *Lythrum salicaria* and *Phragmites australis* were only present in winter samples.

A small number of species that germinated from the samples could be categorized as specialist fen plants within a UK context (Rodwell 1991; e.g. *Berula erecta*, *Juncus subnodulosus*, *Carex panicea*, *Galium uliginosum*), whilst the majority of germinants were generalists known from a variety of other UK grassland or woodland habitats (e.g. *Calamagrostis epigejos*, *Juncus bufonius*, *Plantago lanceolata*, *Urtica dioica*). However, a vegetation classification using all species present in the dung resulted in close affinities to a *Cirsio dissecti-Molinietum* fen meadow assemblage.

Traits for background vegetation and dung samples are summarized in Table 2. There were significant ( $P = <0.05$ ) differences between dung and background vegetation for

**Table 1.** Plant species recorded from the background vegetation and plant species that germinated from Konik dung samples. Table headings: A = DOMIN score (background vegetation); B = Mean dung seed abundance  $\text{kg}^{-1}$ ; C = Number of dung samples in which a species occurred month $^{-1}$ ; D = Peak abundance (month of the year) i.e. Jan = 1, Dec = 12. Species in the background vegetation that were estimated at  $\geq 4\%$  cover across the 56-ha study area are listed, together with species estimated as  $\leq 4\%$  cover but categorized as constituent species of a main fen vegetation type (Rodwell 1991) and/or species that germinated from the dung samples. DOMIN scale: 1 =  $<4\%$  (few individuals); 2 =  $<4\%$  (several individuals); 3 =  $<4\%$  (many individuals); 4 = 4–10%; 5 = 11–25%. Cover/abundance for an individual species did not exceed 25%. Mean abundance for dung samples is measured as number of seeds  $\text{kg}^{-1}$  dry dung. The number of dung samples in which a species occurred/month had a maximum value of  $n = 12$ . Peak abundance refers to the sample month with the highest recorded number of seedlings per species. Abbreviations: sb. = present as a shrub in the background vegetation; t. = present as a tree in the background vegetation.

Species	A	B	C	D
<i>Agrostis stolonifera</i>	5	27.6	11	8
<i>Angelica sylvestris</i>	1	–	–	–
<i>Berula erecta</i>	2	12	2	4
<i>Calamagrostis canescens</i>	5	–	–	–
<i>Calamagrostis epigejos</i>	4	1.7	3	9
<i>Calystegia sepium</i>	3	–	–	–
<i>Carex disticha</i>	2	3.7	3	8
<i>Carex flacca</i>	1	4.7	3	7
<i>Carex hostiana</i>	2	–	–	–
<i>Carex lepidocarpa</i>	2	–	–	–
<i>Carex otrubae</i>	3	3.3	3	7
<i>Carex panicea</i>	2	2.5	4	8
<i>Carex riparia</i>	1	–	–	–
<i>Centaurea nigra</i>	1	–	–	–
<i>Cirsium dissectum</i>	2	–	–	–
<i>Cirsium palustre</i>	2	–	–	–
<i>Cladium mariscus</i>	4	–	–	–
<i>Dactylorhiza incarnata</i>	1	–	–	–
<i>Deschampsia cespitosa</i>	2	–	–	–
<i>Epilobium ciliatum</i>	1	2.5	2	8
<i>Epilobium hirsutum</i>	3	4	4	10
<i>Epilobium tetragonum</i>	1	1	2	12
<i>Eupatorium cannabinum</i>	3	1.8	4	10
<i>Filipendula ulmaria</i>	2	–	–	–
<i>Galium palustre</i>	3	–	–	–
<i>Galium uliginosum</i>	2	2.3	3	1
<i>Glechoma hederacea</i>	3	–	–	–
<i>Glyceria declinata</i>	1	3	1	12
<i>Glyceria fluitans</i>	1	2	2	12
<i>Hydrocotyle vulgaris</i>	2	–	–	–
<i>Iris pseudacorus</i>	3	–	–	–
<i>Juncus articulatus</i>	2	97.3	9	8
<i>Juncus bufonius</i>	2	63.6	8	8
<i>Juncus effusus</i>	1	2.5	2	9
<i>Juncus inflexus</i>	3	18.9	9	8
<i>Juncus subnodulosus</i>	4	10.9	8	9
<i>Lathyrus palustris</i>	1	–	–	–
<i>Linum catharticum</i>	2	–	–	–
<i>Luzula multiflora</i>	1	2	1	9

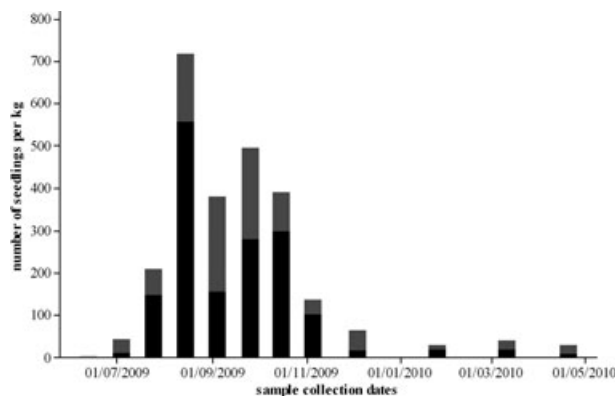
**Table 1.** (Continued).

Species	A	B	C	D
<i>Lycopus europaeus</i>	2	4.5	2	1
<i>Lysimachia vulgaris</i>	2	–	–	–
<i>Lythrum salicaria</i>	2	3	1	12
<i>Mentha aquatica</i>	3	2.7	3	10
<i>Molinia caerulea</i>	4	–	–	–
<i>Myosotis laxa</i>	1	–	–	–
<i>Phalaris arundinacea</i>	4	–	–	–
<i>Phleum pratense</i> s. str.	2	1	1	10
<i>Phragmites australis</i>	4	5	1	12
<i>Plantago lanceolata</i>	1	2.5	2	8
<i>Plantago major</i>	1	14.1	7	8
<i>Poa annua</i>	1	3.3	3	7
<i>Poa pratensis</i>	1	2.3	4	8
<i>Poa trivialis</i>	4	11.6	5	8
<i>Polygala vulgaris</i>	1	–	–	–
<i>Populus canadensis</i> (sb.; t.)	1	1.5	2	7
<i>Potentilla erecta</i>	1	–	–	–
<i>Potentilla reptans</i>	2	4	1	9
<i>Primula veris</i>	1	–	–	–
<i>Prunella vulgaris</i>	2	2.6	7	9
<i>Ranunculus flammula</i>	2	–	–	–
<i>Rhinanthus minor</i>	3	–	–	–
<i>Salix cinerea</i> (sb.)	3	1.5	2	7
<i>Salix repens</i>	3	–	–	–
<i>Samolus valerandi</i>	2	5.2	6	8
<i>Scrophularia auriculata</i>	2	–	–	–
<i>Scutellaria galericulata</i>	3	–	–	–
<i>Silene flos-cuculi</i>	2	–	–	–
<i>Solanum dulcamara</i>	2	5	2	10
<i>Succisa pratensis</i>	2	–	–	–
<i>Symphitum officinale</i>	3	–	–	–
<i>Thalictrum flavum</i>	2	–	–	–
<i>Thelypteris palustris</i>	2	–	–	–
<i>Thyselium palustre</i>	1	–	–	–
<i>Trifolium pratense</i>	1	2	2	12
<i>Triglochin palustre</i>	1	–	–	–
<i>Typha latifolia</i>	2	3	1	7
<i>Urtica dioica</i>	2	45.5	4	9
<i>Valeriana dioica</i>	2	–	–	–
<i>Valeriana officinalis</i>	2	–	–	–
<i>Veronica beccabunga</i>	2	6	2	9
<i>Veronica catenata</i>	1	4.3	3	7
<i>Vicia cracca</i>	3	–	–	–

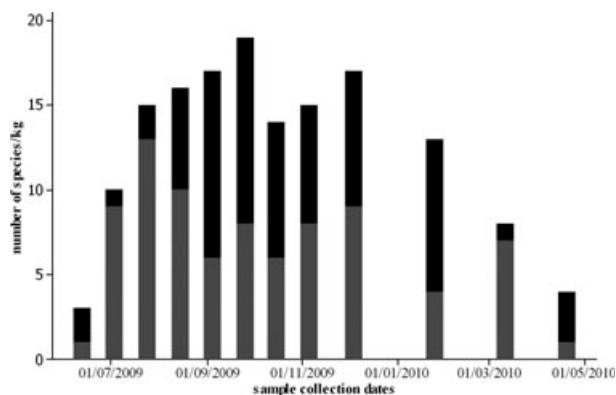
six of the 13 traits measured. Mean values for seed release heights (minimum and maximum) and dispersule weight were significantly lower in species found in the dung, whilst dung species had significantly higher seed bank persistence, inferred Ellenberg N values, and had significantly greater numbers of seeds per shoot.

## Discussion

Almost one-third of the available species pool was present in Konik dung samples, a value which is similar to that



**Fig. 1.** Mean number of germinable seeds  $\text{kg}^{-1}$  (dry weight) from dung samples collected Jun 2009–May 2010. Black shaded areas represent the number of seedlings belonging to the *Juncaceae*.



**Fig. 2.** Total number of plant species per collection date dry  $\text{kg}^{-1}$  of Konik dung. Black shaded area represents the total number of species that germinated from each dung samples. Grey shaded area represents the number of graminoid species that germinated from each sample.

found in previous studies in other habitats (e.g. Malo & Suárez 1995; Traba et al. 2003; Kuiters & Huiskes 2010), and demonstrates the potential importance of endozoochorous dispersal for plants found in temperate fens. It is possible that suboptimal germination conditions for some species in the seedling emergence study or species rarity in the background vegetation led to an underestimation of plants capable of surviving internal transportation.

Based on our seedling emergence results, free-roaming horses have the ability to transport hundreds of thousands of seeds in each calendar year (see also Cosyns et al. 2005; Jaroszewicz et al. 2009; Kuiters & Huiskes 2010), and have the potential to disperse the component species of a range of vegetation types recorded within the study area. The high proportion of viable graminoid seeds recorded from the samples reflects similar results from other endozoochorous studies in other habitats (Welch 1985; Dai 2000;

**Table 2.** Results of analysis of plant traits (overall mean values) for species recorded in the standing vegetation but absent from dung samples and species recorded in the Konik dung, using one-sample *t*-tests. Mean values for each trait for species germinating from dung samples were calculated by presence of species in each sample ( $n = 12$ ) and the associated trait value. Background vegetation trait values were calculated from the presence of species recorded in the vegetation but absent from dung samples and the associated trait value. Dispersule weight: 1 =  $\leq 20$  mg; 2 = 0.21–0.50 mg; 3 = 0.51–1.00 mg; 4 = 1.01–2.00 mg; 5 = 2.01–10.00 mg. Dispersule shape is based on length/breadth ratio, where 1 =  $\leq 1.5$ ; 2 = 1.5–2.5; 3 =  $\geq 2.5$ . Ellenberg L (light): 5 = plants of semi-shade, rarely in full light; 6 = intermediate between 5 and 7; 7 = plants of generally well lit places. Ellenberg N (nutrient): 3 = infertile sites; 4 = intermediate between 3 and 5; 5 = sites of intermediate fertility; 6 = intermediate between 5 and 7; 7 = rich, fertile soils. Ellenberg F (moisture): 5 = average dampness; 6 = intermediate between 5 and 7; 7 = constantly moist or damp soils. Seed bank longevity index ranges from short-lived (0) to long-lived (1).

Trait	Background vegetation (not present in dung) mean trait score	Konik dung mean trait score	<i>P</i> -value	<i>t</i>
Dispersule weight (mg)	3.16	2.08	<0.001	–14.42
Dispersule shape (length/breadth ratio)	1.80	1.8	0.974	0.03
Ellenberg light (L)	7.07	7.05	0.808	0.25
Ellenberg nitrogen (N)	4.94	5.48	0.017	2.80
Ellenberg moisture (F)	6.90	7.38	0.096	1.82
Competitor value (C)	0.40	0.45	0.060	2.10
Stress-tolerator value (S)	0.26	0.24	0.204	–1.35
Ruderal value (R)	0.34	0.31	0.938	–0.08
Seed bank longevity	0.25	0.48	<0.001	12.30
Seed release height (cm) min	50.34	26.49	<0.001	–13.95
Seed release height (cm) max	158.09	86.31	<0.001	–19.62
Seed number per shoot	6073.00	32420.55	0.043	2.29

Mouissie 2004; but see Malo & Suárez 1995). However, germination and establishment of internally dispersed seeds under natural field conditions, post-dispersal, is likely to be substantially lower than seedling emergence results suggest, as they are affected by a number of biotic and abiotic filters. These include seed predation (e.g. Janzen 1971), modified hydrology or soils (e.g. Willis & Mitsch 1995), a deficiency of species-specific germination cues and competition from existing species in the sward (e.g. Pywell et al. 2002; Hobbs & Norton 2004), although competition may initially be moderated by the suppression of vegetation resulting from deposition of seeds in a dung package. An example of the disparity between controlled seedling emergence results and natural outcomes is given in a study undertaken by Cosyns et al. (2006) in which less than 5% of the high number of viable monocotyledon seeds recorded in seedling emergence studies successfully established in the field after a 3-mo period. In contrast, although their study found that low numbers of dicotyledon seeds were dispersed, they achieved a higher sward cover and contributed to greater species richness in natural conditions after 1 yr. Consequently, caution should be used regarding the interpretation of results due to considerable differences between glasshouse and field conditions (Eichberg et al. 2007).

The highest number of species per sample occurred in late Sep, coinciding with the conclusion of flowering and peak seed production for the majority of species within the background vegetation (cf. Stace 2010). The relationship

between seed production and dung seed density has previously been demonstrated in studies investigating endozoochorous dispersal in calcareous grasslands (Bakker & Olff 2003) and heathland (Mouissie et al. 2005). Following this peak, the number of species recorded in the dung falls away, before briefly rising to a second peak in Dec. The fall in the number of species recorded between these two peaks may be due to seed mortality or to seed fall from species that retain fruits on the parent plant for only a short period of time, combined with a temporary increase in the abundance of palatable species with seed traits that are not conducive to endozoochorous dispersal. The second species abundance peak that then follows in the winter could be linked to a reduced palatable vegetation resource and the browsing of species that retain seeds intact on the parent plant for a prolonged period of time. The presence of relatively high numbers of species in the Dec sampling date points to the importance of collecting samples outside of the main growing season, particularly as three of the species that were only recorded in the winter months of our study were strongly associated with wetland or fen vegetation. Mouissie et al. (2005) also found that two constituent species of heathland vegetation (*Calluna vulgaris* and *Erica tetralix*) were only dispersed in the winter months.

Plant trait results demonstrated that there were a wide range of characteristics that are conducive to internal dispersal, supporting Janzen's (1984) central hypothesis. Species of fen vegetation dispersed via endozoochory had a more persistent seed bank than species that were not dis-

persed, had higher seed production rates and may also establish in sites of higher fertility. These traits could be viewed as advantageous for dispersal through dung, considering the high level of nutrients contained within the growing medium, the subsequent creation of areas of bare ground and initial low levels of competition from the surrounding vegetation. For example, producing large numbers of small seeds would increase opportunities for consumption by unselective grazing, and the recruitment opportunities created by grazing disturbance would favour species that rely on an open sward and minimal competition for germination and establishment.

Pakeman (2001), Pakeman et al. (2002) suggested that the traits that are necessary to survive ingestion by animals are similar to those that permit long-term survival in the soil. Our data, together with previous seed bank work undertaken at the study site (Stroh et al. in press), support this supposition. It may be that similarities between dispersal strategies reflect traits that are necessary to survive being interred within another medium (e.g. gut, dung or soil) for a prolonged period of time. In contrast, alternative 'external' dispersal mechanisms (epizoochory) by grazing animals are more likely to transport short-lived seeds, and in doing so complement species dispersed by endozoochory (Couvreur et al. 2004).

The two background species categorized as 'rare' in a UK context (*Lathyrus palustris* and *Thyselium palustre*) were not identified in the dung samples. One explanation for this absence may be simply that the seeds of these species were not consumed due to the large size of the study area and/or the low numbers of individual plants in the background vegetation. Alternatively, it may be that the seeds of these species were consumed but did not survive the digestive process due to associated functional traits. As stated earlier, one explanation for the inability to survive the digestive process may be linked to the length of time a seed can survive as a viable germinant in the soil seed bank. Both species are known to have transient (<1 yr survival) seed banks (Thompson et al. 1997), and this deterrent to internal dispersal may also be applicable to other temperate fen species (e.g. *Cirsium dissectum*, *Carex lepidocarpa*, *Cladium mariscus*, *Succisa pratensis*, *Rhinanthus minor*) found in the current study, which are also known to have a transient seed bank (e.g. Thompson et al. 1997; Matus et al. 2003). In the cases of *L. palustris* and *T. palustre*, long-distance dispersal strategies are more likely to be adapted to hydrochorous dispersal (see Vaughan 1978; Meredith & Grubb 1993).

Seed weight was significantly lower for dung-dispersed species, but seed shape was not significantly different from that of non-dispersed species, with the mean dimensions suggesting a shape that was more elongate than rounded. This was somewhat surprising, as many studies have

concluded that small, rounded seeds are adapted to endozoochorous dispersal (e.g. Bruun & Fritzbøger 2002; Pakeman et al. 2002; but see Cosyns & Hoffmann 2005). However, the mean seed weight for dispersed species does describe a small and very light seed. Such an inconspicuous size is an advantage during the digestive process as large and heavy diaspores are more likely to be damaged by the molar mill (Murphy et al. 1989). Species that produce small and light seeds are also more likely to produce greater quantities of seed (Jakobsson & Eriksson 2000) and in doing so increase the probability of dispersal potential from the parent plant (Bruun & Poschod 2006). The results of our analysis with regard to the marginally but still significantly greater number of seeds produced per shoot for sample species support this assertion.

The significant difference between dung samples and background vegetation found for seed release heights may be explained by at least two factors. First, the lower minimum and maximum seed release heights for endozoochorous species are likely to be linked to the grazing strategies of large herbivores, and specifically in this instance Koniks, which preferentially graze a shorter sward because of the higher nutritional value of young plant growth (van Braeckel & van Looy 2002; but see Naujeck & Hill 2005). This in turn would inadvertently result in the unselective ingestion of plants and seeds at the same height in the surrounding vegetation as well as those selected for consumption. Second, seeds of plants that have a relatively tall mean height in the sward are more likely to have an epizoochorous dispersal strategy (e.g. Couvreur et al. 2005) or an anemochorous dispersal strategy (e.g. Davies & Sheley 2007).

Historically [from around 2,500 yrs ago when the most recent fen peats were formed in East Anglia (Godwin 1939) until fen drainage during the 17th century], wild herbivores would have browsed floodplain fens, but their abundance, and hence impact, is likely to have been greatest at the floodplain margins where there would have been firmer ground for resting and, for some species, creation of latrines. This combination of increased grazing intensity and nutrient return through dung could have resulted in the development of vegetation assemblages containing many species that are now considered typical of 'fen meadows'. As human beings began to exploit the wetlands, their impact too would have been greatest at the floodplain margin, creating mowing meadows and herding their livestock on the shallower peat. In situations such as those found at the study site, where NNR vegetation is adjacent to large-scale habitat restoration land, reinstating free-roaming herds that are able to roam from NNR vegetation onto ex-arable restoration land could re-establish previously lost dispersal links through the landscape. Free-roaming grazing animals thus have the potential to be an

effective tool for diversifying restoration vegetation, particularly in areas that share similarities to floodplain margins, such as wetland drawdown zones.

## Conclusions

The loss of landscape dispersal mechanisms has been directly associated with the loss of plant diversity in northwest Europe in the past 100 yrs (Ozinga et al. 2009). In this context, free-roaming grazing animals used as mobile links between wetland and temperate fen plant populations have the potential to become important vectors in helping to maintain or restore vegetation through natural colonization and increased gene flow between extant populations. This study has demonstrated that Koniks may disperse the viable seeds of a wide range of temperate fen species throughout the year, and indicates that optimum times for the movement of grazing animals and potential dispersal of germinable seeds are not restricted simply to the growing season. The conservation grazing system at Wicken Fen NNR and the Wicken Vision both have year-round grazing systems with free-roaming Koniks. If a link is created between the NNR and the restoration land, then free-roaming grazing herds have the potential to be an effective tool for diversifying restoration vegetation that, at the Wicken Vision site, is developing through natural regeneration and hydrological manipulation.

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