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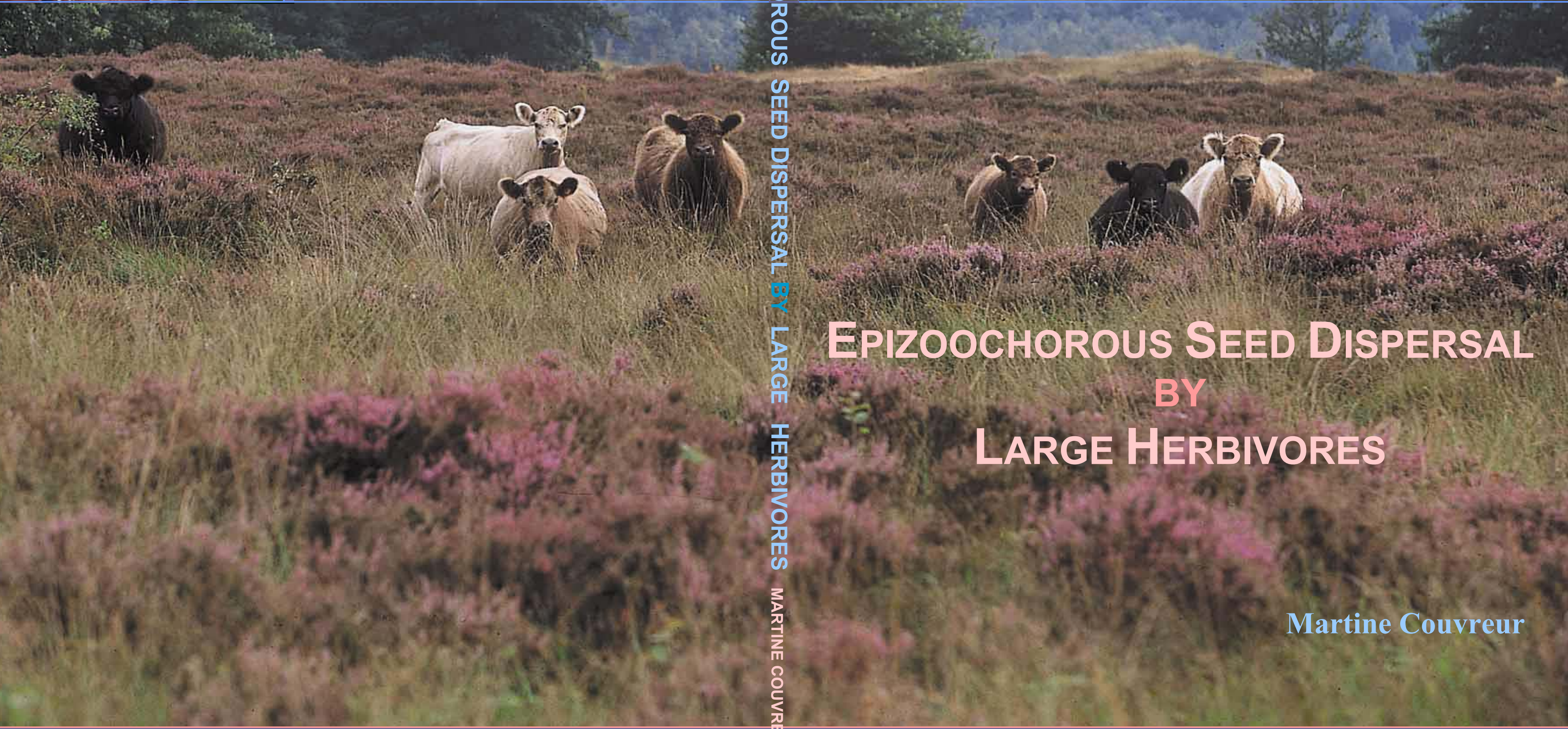
2005 EPIZOOCHOROUS SEED DISPERSAL BY LARGE HERBIVORES MARTINE COUVREUR



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Martine Couvreur

Faculteit Bio-ingenieurswetenschappen

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EPIZOOCHOROUS SEED DISPERSAL BY LARGE HERBIVORES

EPIZOÖCHORE ZAADVERBREIDING DOOR GROTE HERBIVOREN

door

Martine Couvreur

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of the requirements for
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Op gezag van: Rector Prof. Dr. A. De Leenheer

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Copromotor: Prof. Dr. M. Hermy, Katholieke Universiteit Leuven
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Samenvatting

De verbreiding van plantenzaden via de vacht van dieren (epizoöchorie) is één van de belangrijke mechanismen die de lange-afstandsverbreiding van planten kunnen mediëren. In vele ecosystemen is zaadverbreiding een 'bottleneck' voor kolonisatie en migratie van planten. In het snel veranderende landschap in Vlaanderen en west-Europa zijn vele (half)natuurlijke habitats bovendien verloren gegaan, en zijn de resterende gebieden sterk versnipperd. De doorgedreven modernisering van de landbouw heeft ook heel wat traditionele gebruiken doen verdwijnen, hetgeen in het algemeen tot een verminderd zaadaanbod en een verminderde connectiviteit van het landschap heeft geleid. Epizoöchorie door in het wild levende zoogdieren en loslopend vee heeft bijgevolg sterk aan belang ingeboet. Anderzijds is begrazing met grote herbivoren momenteel één van de belangrijkste natuurbeheerpraktijken geworden, waarbij regelmatig transport van vee komt kijken. Via deze grazers kan dus potentieel nog een belangrijke uitwisseling van zaden tussen soortenrijke gebieden gebeuren. Lange-afstandszaadverbreiding is echter moeilijk te traceren, zeker wanneer er complex diergedrag bij komt kijken. Ondanks het toenemend aantal onderzoeken naar zaadverbreiding is epizoöchorie dan ook nog weinig gekend.

In deze studie worden verschillende aspecten van epizoöchorie belicht, via beschrijvend, experimenteel en modelleringsonderzoek. De voornaamste doelstellingen waren (1) inzicht verwerven in de hoeveelheid, identiteit en eigenschappen van zaden die via epizoöchorie verbreid worden, (2) belangrijke aspecten van epizoöchorie experimenteel kwantificeren, en (3) velddata rond epizoöchorie integreren in een model. Hiertoe bestudeerden we vooral grote grazers die ingezet worden voor de begrazing in Vlaamse natuurgebieden. Deze kunnen fungeren als model voor wilde zoogdieren in verleden, heden en toekomst, zijn relatief gemakkelijk te onderzoeken, en spelen een cruciale rol in het hedendaags natuurbeheer in onze streken.

In een eerste deel van deze studie werden de vachten van een 200-tal grote herbivoren (Galloway-runderen, paarden en ezels) uit Vlaamse natuurgebieden geïnspecteerd op zaden. We identificeerden 6385 kiemkrachtige zaden van 75 plantensoorten, met een aanzienlijke variatie aan planteigenschappen en zaadmorfologieën. In tegenstelling tot wat oorspronkelijk werd verondersteld, worden dus niet alleen zaden met morfologische aanpassingen voor epizoöchorie (bv. haakjes) verbreid in de vacht van dieren, maar ook ongespecialiseerde zaden en zaden aangepast aan andere verbreidingsmechanismen (bv. wind, water). Onze resultaten geven aan dat de rol van grote herbivoren in zaadverbreiding aanzienlijk is, en dat

zij als ‘mobiele links’ binnen en tussen habitats fungeren -bij diertransporten in het kader van seizoensbegrazing zelfs tussen natuurgebieden op grote afstanden.

Dieren verbreiden niet enkel zaden via epizoöchorie, maar ook via endozoöchorie (na doorgang van de zaden door het spijsverteringsstelsel). Beide mechanismen opereren gelijktijdig, maar werden nog maar zelden vergeleken als gevolg van hun mechanistische verschillen. Bij onderzoek naar de relatie tussen epi- en endozoöchorie vonden we dat in een kustduinengebied 20% van de lokale plantensoorten via de grote grazers werd verbreid, met seizoenale verschillen in soortensamenstelling. Een complementaire set aan plantensoorten werd verbreid via epi- respectievelijk endozoöchorie (slechts één vierde van de 66 zoöchore soorten werd verbreid door beide mechanismen). Deze complementariteit werd bevestigd op niveau van verbreidingsgerelateerde plantkenmerken, die gebruikt werden om de soorten in het studiegebied op te delen in functionele planttypes. Een vergelijking van het aandeel epi- en endozoöchoor verbreide soorten in deze functionele groepen gaf aan dat epizoöchorie specifieker was dan endozoöchorie (geassocieerd met een kleiner aantal functionele plantgroepen). De resultaten tonen aan dat epi- en endozoöchorie beide belangrijk zijn, als additieve en complementaire verbreidingsmechanismen.

Een sleutelaspect van epizoöchorie is de adhesieve interactie tussen zaden en vachten. In een tweede, experimenteel luik, kwantificeerden we deze interactie met behulp van gelooide vachten van gedomesticeerde en wilde dieren, via een eenvoudige gestandardiseerde methode. Op die manier bekwamen we een maat voor de epizoöchore verbreidingsmogelijkheden van uiteenlopende zaadsoorten in relatie tot verschillende vachten. Diepe vachten met lange, golvende haren en een verticale haarinplant (Galloway-rund, schaap, wild zwijn) waren beter geschikt voor de aanhechting van zaden dan ondiepe vachten met korte, gladde haren die horizontaal ingeplant zijn (ree, Holstein rund, konijn, paard). In het algemeen werd de zaadaanhechting gefaciliteerd door gespecialiseerde zaadaanpassingen (haken, borstels, kafnaalden, haren, …) en bemoeilijkt door niet-gespecialiseerde aanpassingen zoals steeltjes of vleugels. Nochtans was de functionaliteit van bepaalde zaadmorfologieën sterk afhankelijk van het type vacht (interactie-effect). Zaadmorfologie bleek een goede predictor te zijn voor zaadaanhechting, alhoewel ook minder adhesieve zaden relatief goed scoorden. Dit suggereert dat zo goed als alle soorten tot op zekere hoogte intrinsiek in staat zijn tot epizoöchore zaadverbreiding.

Naast de loutere interactie tussen zaden en vachten beïnvloeden ook de omgeving en het diergedrag de retentietijd en de potentiële verbreidingsafstand van zaden in vachten. Daarom maten we de retentietijden van gemarkeerde zaden in de vacht van twee soorten grote herbivoren in veldcondities. In de lange vacht van Galloway-runderen werden de zaden significant langer meegevoerd dan in de korte vacht van Haflinger-paarden. De structuur van de omringende vegetatie had geen eenduidig effect op de retentietijden van de zaden, en regen had een verwaarloosbare invloed. Via regressie-analyse konden halfwaarde-retentietijden van de geteste zaden berekend worden, die tot meer dan 4 uur voor Haflinger-paard en tot meer dan 12 uur voor Galloway-rund bedroegen. De corresponderende potentiële verbreidingafstanden waren in de grootteorde van tientallen meters tot kilometers. Zaadafmetingen en adhesieve zaadaanpassingen beïnvloedden de zaadretentie in de lange rundervacht en in de korte paardenvacht op een verschillende manier.

Tenslotte integreerden we verschillende veldgegevens in een ruimtelijk expliciet simulatiemodel, dat de belangrijke aspecten van epizoöchorie omvat. Het model werd toegepast op een 80 ha groot kustduinengebied, en de parameters werden bepaald op basis van empirische studies naar de beweging en het habitatgebruik van de ezels in het gebied, en op basis van gegevens over de distributie, zaadproductie, zaadtoegankelijkheid, zaadaanhechting en zaadretentie van een aantal epizoöchore plantensoorten. Ter validatie van het model vergeleken we voorspelde met geobserveerde zaadaantallen in de ezelvacht. De gemodelleerde zaadschaduwen toonden aan dat voor de meeste soorten ongeveer de helft van de zaden over een netto-afstand van meer dan 100 m verbreid wordt door de ezels, en ongeveer 1% verder dan 800 m. Het model kon de variatie tussen de verschillende plantensoorten qua geobserveerde zaadaantallen in de vacht verrassend goed voorspellen, zeker gezien de relatief ruwe inschattingen van zaadproductie en zaadbereikbaarheid voor de dieren. Het model bevestigt de belangrijke rol van epizoöchorie in het mediëren van lange-afstandsverbreiding, en voorziet een modelleringskader om de veelvoudige componenten van het verbreidingsproces te integreren.

Uit deze studie blijkt dat epizoöchorie een efficiënt verbreidingsmechanisme kan zijn voor een brede waaier aan plantensoorten, vooral door langharige diersoorten of rassen. Doorgaans zijn de verbreidingsafstanden ook voldoende groot om geïsoleerde habitats in gefragmenteerde landschappen te kunnen verbinden. Als ‘mobiele link-organismen’ kunnen grote herbivoren bijgevolg belangrijke instrumenten zijn in ecologische restauratieprojecten waar zaadverbreiding een beperkende factor is voor vegetatieontwikkeling.

Summary

The dispersal of plant seeds in the fur of animals (termed epizoochory) is one of the important mechanisms effectuating long-distance dispersal of plants, a crucial aspect of plant spatial dynamics. Several ecosystems in the world are dispersal-limited, pointing to long-distance seed dispersal as a bottleneck for plant colonization and migration. In the rapidly changing landscape of Flanders and western-Europe, many (semi-)natural habitats have disappeared, and the remaining ones are highly fragmented. In addition, the modernization of agricultural practices has led to a decreased availability of seeds in the landscape and a declined landscape connectivity. Hence, epizoochory by wild mammals and free-ranging livestock has lost importance. On the other hand, grazing by large herbivores is currently one of the most frequently used nature management practices, involving animal transports over large distances. These grazers may still mediate an important seed exchange between species-rich habitats. However, long-distance seed dispersal is intrinsically difficult to trace, especially when complex-behaving animals are involved. As a consequence, epizoochory is still poorly understood, despite the increasing number of studies devoted to seed dispersal.

In this study, we investigate several aspects of epizoochory, using descriptive, experimental and modelling approaches. Our main objectives were (1) to gain insight in the quantity, identity and characteristics of seeds dispersed by epizoochory, (2) to experimentally quantify important aspects of epizoochory, and (3) to create a backbone for integration of field data into a model of epizoochory. To reach these goals, we mainly focused on large herbivores used for grazing in nature reserves, as they can serve as models for wild mammals in the present, past and future, they are relatively easy to examine, and play a major role in contemporary nature management in our regions.

First, we inspected epizoochorous seed loads of a large number of herbivores (Galloway cattle, horses and donkeys) in Flemish nature reserves. Examination of the species and plant traits showed that a wide variety of species (75 in total), with a broad range of seed morphologies and plant characteristics, can disperse by epizoochory. In contrast to what was traditionally assumed, not only seeds with morphological adaptations to epizoochory (e.g. hooks, bristles) were observed, but also unspecialized seeds and seeds adapted to other dispersal mechanisms (wind, water). Our results suggest that the seed dispersal role of large herbivores in nature reserves may be considerable, as they act as ‘mobile links’ within and between habitats. Seed dispersal was even observed between isolated nature reserves at large distances, when large herbivores were transported between reserves in the context of seasonal grazing projects.

Animals do not only disperse seeds via epizoochory, but also via endozoochory (after passage through the digestive tract). Both mechanisms operate simultaneously, but have been rarely compared as a consequence of their mechanistic differences. Therefore, we investigated the relationship between epi- and endozoochory, focusing on free-ranging donkeys in a coastal dune ecosystem. In total, 20% of the species recorded in the study area were observed to be dispersed by zoochory, with seasonal differences in species composition. We observed a complementary set of plant species dispersed by epi- and endozoochory (only one fourth of the 66 zoochorous species were dispersed by both mechanisms). This complementarity was confirmed on the level of dispersal-relevant plant traits, which were used to define dispersal-functional plant types among all species in the study site. Comparison of the portion of epi- or endozoochorously dispersed species in these functional groups revealed that epizoochory was more specific than endozoochory, and was associated with a more narrow range of dispersal-functional plant types. The results stress the importance of both epi- and endozoochory, as additive and complementary dispersal mechanisms.

Next, we experimentally quantified the adhesive interaction between seeds and furs -a key aspect of epizoochory- using prepared furs of domesticated and wild animals and a simple standardized method. As such, a measure of epizoochorous dispersal potential was obtained for 66 seed species with respect to the different furs. Deep furs with long, rough, undulated hairs implanted at a large angle (Galloway cattle, sheep, wild boar) proved most suited for seed adhesion, in contrast to shallow furs with short, smooth, straight hairs implanted at small angles (roe deer, Holstein cattle, rabbit, horse). Generally, the adhesivity of seeds was facilitated by specialized adhesive appendages, yet hampered by unspecialized seed appendages, which led to lower adhesivity scores than a lack of appendages. Still, an interaction-effect between certain seed and fur types was noticed. Although seed morphology was a good predictor for seed adhesivity on fur, less-performing seed types often still had relatively high adhesivity scores. This suggests that nearly all species are to some extent intrinsically able to disperse epizoochorously.

Besides the mere interaction between seeds and furs, animal behaviour and environment influence the retention time and potential dispersal distances of seeds in fur. Therefore, we quantified retention times of marked seeds in fur of large herbivores in field conditions. In the long fur of Galloway cattle, seeds were retained significantly longer than in the short fur of Haflinger horse. In general, seed retention times were not considerably affected by the structure of the surrounding vegetation, and negligible differences were observed between dry and wet furs. Using regression analysis, half-life seed retention times could be calculated, which mounted to 4 hours for Haflinger horse and to 13 hours for

Galloway cattle. The corresponding potential half-life dispersal distances were in the order of magnitude of tens of metres to a few kilometres. Seed traits correlated differently with seed retention times in the long cattle fur and in the short horse fur, respectively.

Finally, we incorporated our field data into a spatially explicit simulation model including the important aspects of epizoochory. The model was applied to a 80 ha coastal dune nature reserve, and parameterized using empirical studies of the movement and behaviour of the grazing donkeys, and data on the distribution of selected plant species, their seed production, seed accessibility, seed adhesion, and seed retention in fur. To validate the model, we compared predicted with observed seed numbers on donkey fur. The modelled seed shadows indicated that for most species about half of all seeds dispersed by donkeys travel a net distance of >100 m, and about 1% travel >800 m within this 80 ha nature reserve. The variation among species in the observed seed numbers found on donkey fur was surprisingly well predicted by the model given the relatively crude estimates of seed production and accessibility to donkeys. The model confirms the important role of epizoochory in effecting long-distance seed dispersal, and provides a modelling framework for integrating the multiple components of the dispersal process.

In conclusion, epizoochory can provide an efficient means of dispersal for a wide range of plants species, over distances sufficient to connect isolated habitats in fragmented landscapes. As ‘mobile link organisms’, large herbivores can therefore be important instruments in ecological restoration projects where seed dispersal is a bottleneck for vegetation development.

Chapter 1

Introduction

Seed dispersal as a key process in plant population ecology

Diaspores[1] (further referred to as seeds) represent the potentially mobile life stage of plants. Despite the possibility of many species to expand vegetatively, plants passively move in space (and time) basically through the dispersal of their seeds. As such, seed dispersal is a major factor driving plant spatial dynamics, both at local and regional scales. Seed dispersal ultimately determines the distribution of plant populations and communities (e.g. Cain et al. 2000, Bullock et al. 2002, Levin et al. 2003, Ozinga et al. 2004), the gene flow between plant populations (Loveless & Hamrick 1984) (together with pollen flow, White et al. 2002), and plant metapopulations dynamics (Eriksson 1996, Cain et al. 2000).

Until recently, the key role of seed dispersal has been underestimated, as it has long been assumed that species distributions (and species-richness) were a function of the abiotic conditions of an ecosystem and that all species can reach all suitable habitats. Of course, the effects of dispersal on plant spatial dynamics are strongly modified by the spatial pattern of abiotic and biotic influences on establishment, growth, and survival (Schupp & Fuentes 1995, Fig 1.1), yet the assumption that the omnipresence of seeds is self-evident has proven wrong. In contrast, ecologists have now discovered that dispersal limitation is a major ecological constraint in many ecosystems of the world, at various scales (e.g. Turnbull et al. 2000, Zobel et al. 2000, Dalling et al. 2002, Foster & Tillmann 2003, Verheyen et al. 2003 a, b). Despite other sources for colonization -such as the seed bank, persistent sapling bank or clonal growth- seed dispersal appears to play a major role in the colonization of newly formed gaps in the vegetation (Bullock et al. 2002).

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[1] Diaspores are plant parts which can develop into new plants (e.g. seeds, fruits, root or shoot fragments, spores). In this study, we use the term ‘seed’ in stead of ‘diaspore’, as we mainly refer to seeds and fruits as the dispersal units of plants.

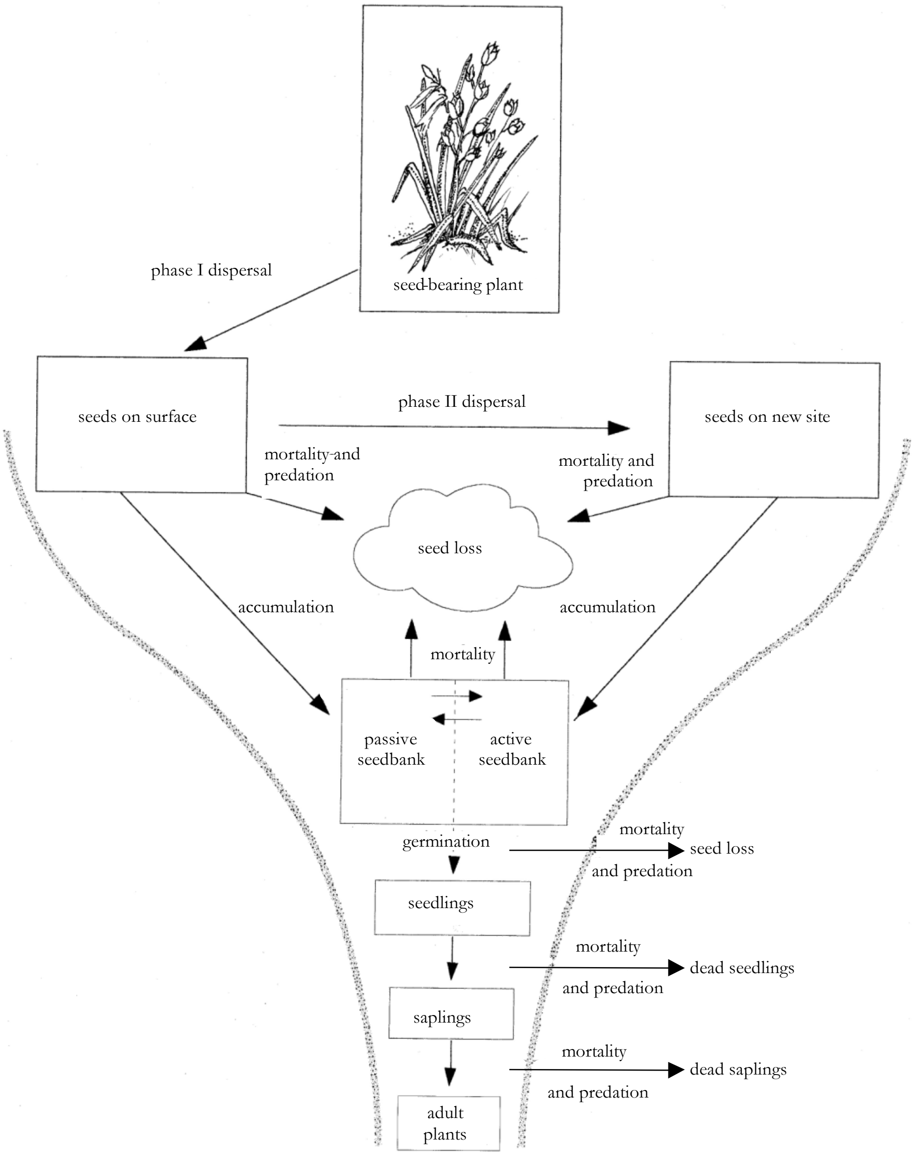

Fig 1.1 *Schematic representation of the colonisation process of plants, showing that dispersal is at the basis of the bottleneck of plant establishment (adapted from Hermy 2004).*

In addition, many recent studies highlighted the crucial role of long-distance[2] seed dispersal in determining range-shifts of plants on a global scale. Understanding spread rates of species is one of the primary motivations for studying dispersal (e.g. Turchin 1998, Okubo & Levin 2001), and models linking dispersal and spread rates have a long history in ecology and evolutionary biology. One of the most puzzling facts in this respect is termed Reid's paradox (Reid 1899, Skellam 1951, Clark et al. 1998), which refers to the discrepancy between estimated Holocene postglacial spread rates of many temperate plants and their observed current dispersal distances. As estimated from pollen records, species must have migrated northwards with average rates in the order of 200 m yr^{-1} following the recession of the glaciers (MacDonald 1993, Clark et al. 1998). However, the dispersal distances ecologists observe in the present typically average no more than a few tens of meters per year for many species (Matlack 1994, Brunet & von Oheimb 1998, Bossuyt et al. 1999), distances which cannot account for the recolonization of northern temperate regions following the glaciers' retreat (Clark et al. 1998). The reason for this discrepancy is that the long-distance component of seed dispersal has largely been underestimated and hence poorly quantified. Whereas most seeds fall near the parent plant, it is the relatively small proportion of seeds that achieves long-distance dispersal (the tail of the dispersal distribution, see Fig 1.2) that is of central importance in the spread of species (Cain et al. 1998, Clark 1998, Clark et al. 1998, Turchin 1998, Higgins & Richardson 1999, Cain et al. 2000, Pakeman 2001).

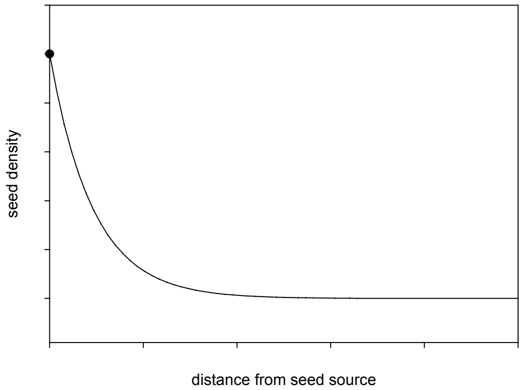

Fig 1.2 *The dispersal curve, showing that most seeds are dispersed near the parent plant, while a small proportion (represented by the tail of the curve) achieves long-distance dispersal.*

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[2] Long-distance seed dispersal can be considered as the passive movement of seeds over distances represented by the tail of the dispersal curve, which is case-specific. Nevertheless, long-distance seed dispersal has been arbitrarily defined by Cain et al. (2000) as dispersal beyond 100 m.

Seed dispersal mechanisms

The great variety of dispersal-aiding morphologies attracted the attention of naturalists as early as Aristotle (384-322 BC) and Theophrastus (371-286 BC) (Thanos 1994). For a long time, the study of seed dispersal was either anecdotal or speculative, with attempts to explain the selective value of every detail of a dispersal unit (Sernander 1906, Ridley 1930, van der Pijl 1982). The most commonly used classification system of dispersal mechanisms is based on the agent or vector of dispersal, typically inferred from seed morphology. The principal agents of dispersal are either abiotic (wind or water) or biotic (animals and the plant itself), and the dispersal modes are termed, respectively, anemochory, hydrochory, zoochory and autochory (van der Pijl 1982, Grime 1988). Among these, zoochory has been identified as an important long-distance dispersal vector (Darwin 1859, Poschlod et al. 1998, Pakeman 2001). Zoochory comprises epizoochory (the external dispersal of seeds on animals) and endozoochory (the internal dispersal of seeds, after passage through the digestive tract of animals). The dispersal of seeds by ants, (myrmecochory, see Sernander 1906) and by scatterhoarding animals (see Hurly & Lourie 1997) are special cases of zoochory and are not considered further here.

However, such classification -using seed morphology to derive the dispersal vector- tends to overlook important characteristics of the seed dispersal process. Dispersal is rarely mediated by a single dispersal agent and is not confined to the primary movement of seeds from the parent plant to the surface (Phase I dispersal, Chambers & MacMahon 1994). Rather, it also entails subsequent movements (Phase II dispersal) that can be mediated by other dispersal agents (see Fig 1.1). In addition, the actual processes responsible for long-distance dispersal appear to be only loosely correlated with those interpreted from seed morphology (Higgins et al. 2003b). The common practice of using the morphological dispersal mode to distinguish short- from long-distance dispersal is therefore questionable.

Seed dispersal by animals: zoochory

As important long-distance dispersal processes, epi- and endozoochory (the external and internal dispersal of plant seeds by animals) have been the subject of an increasing number of observational and experimental studies. We briefly introduce both dispersal modes, mainly focusing on epizoochory.

Epizoochory

Epizoochory refers to adhesive seed dispersal by animals. Although seeds can adhere to various body parts of all sorts of animals (a.o. feathers or feet of birds, Yumoto 1999; hooves of ungulates, Stender et al. 1999), in this thesis we use epizoochory in the context of adhesive seed dispersal in mammalian fur. The first fossil evidence of epizoochory- an awned seed of the grass genus *Pharus* in association with mammalian hair- was discovered in Dominican Republic amber and dates from the Late Eocene (Poinar & Columbus 1992). Seed dispersal in animal fur has been studied on hares by Agnew & Flux (1970) and Sorensen (1986), on mice by Lacey (1981) and Kiviniemi & Telenius (1998), on sheep and goat by Shmida & Ellner (1983), on sheep by Fischer et al. (1996) and Mouissie (2004), on cattle and fallow deer by Kiviniemi (1996), on cattle by Stender et al. (1997) and Kiviniemi & Eriksson (1999), on wild boar by Mrotzek et al. (1999), on wild boar and roe deer by Heinken et al. (2001) and Heinken & Raudnitschka (2002), on wild boar, roe deer, fallow deer and red deer by Schmidt et al. (2005), and on dog by Heinken (2000) and Graae (2002). Although these studies have resulted in an increased insight in the process of epizoochory (see Fig 1.3), many ambiguities and questions remain.

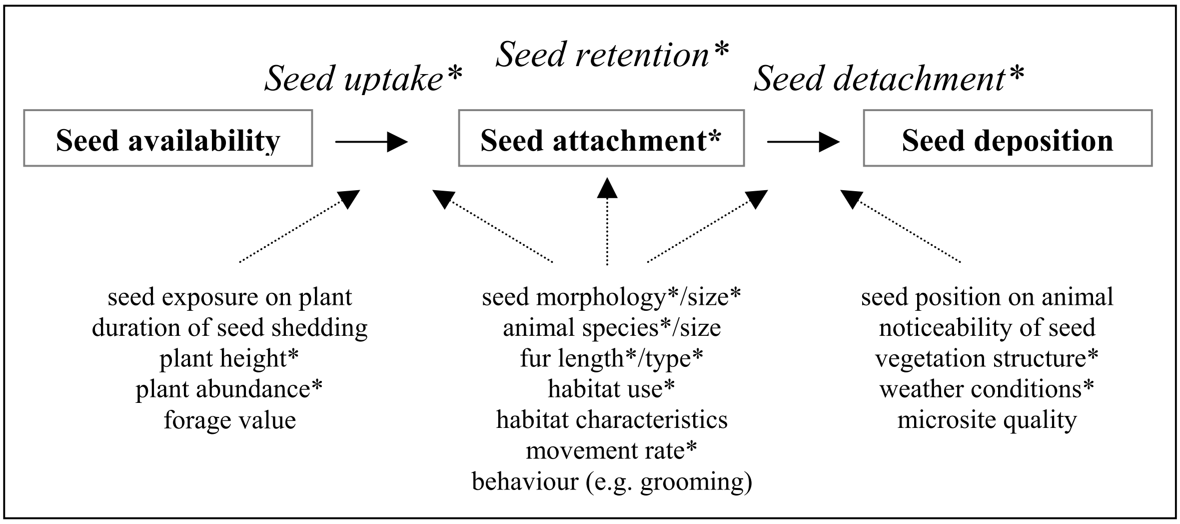

Fig 1.3 *Plant-, animal- and habitat-related factors influencing the consecutive phases of epizoochory (adapted from Wang & Smith 2002). Asterixes indicate aspects studied in this thesis.*

Whereas epizoochory was traditionally associated with morphological seed adaptations facilitating attachment to mammalian fur (such as hooks, bristles, hairs, awns) or mucus-secreting seeds (e.g. *Prunella vulgaris*, *Plantago* spp.), some field studies suggest that epizoochory is not confined to such adapted seeds. While Heinken (2000) and Agnew & Flux (1970) found almost exclusively adhesive species on a dog and on hares, Graae (2002) also reported adhesive seeds and small seeds from tall plants (at least 80 cm) in a dog's fur. Mrotzek et al. (1999) even suggested a negative effect of the presence of adhesive properties on the dispersal frequencies by wild boar, as they observed the majority of seeds in fur being small, smooth diaspores! Most authors emphasize that adhesive adaptations aid epizoochory but are not necessary, given the observation of seeds with and without adhesive adaptations in animal fur (Shmida & Ellner 1983, Fischer et al. 1996, Graae 2002, Heinken & Raudnitschka 2002). Kiviniemi and Telenius (1998) proposed the existence of a trade-off between seed size and effectiveness of epizoochorous dispersal. They argue that large epizoochorous propagules often appear to carry more elaborate appendages than small propagules, to ensure that they adhere efficiently to fur in spite of their size. On the other hand, experimental studies have shown that adhesive seed appendages can be irritating to animals, resulting in an increased grooming behaviour (fur cleaning) and hence an accelerated seed loss (Sorensen 1986, Kiviniemi, 1996). Also the size of the seeds and their noticeability for animals was shown to induce active seed removal (Sorensen 1986). Besides seed morphology, also other factors are likely to influence epizoochory. The height of seed presentation on plants has been reported to be positively related to the frequency of epizoochorous dispersal (Bullock & Primack 1977, Fischer et al. 1996, Stender et al. 1997, Graae 2002), as has the abundance of the species in the local vegetation (Fischer et al. 1996, Stender et al. 1997). Müller-Schneider (1983) and Fischer et al. (1996) also stressed the importance of the duration of the disseminating period. A seasonal variation in epizoochorous species composition could be observed for higher plants (Fischer et al. 1996), but not for moss fragments (Heinken 2000).

The role of fur properties in epizoochorous seed dispersal has been stressed by Lacey (1981), Schmida & Ellner (1983), Kiviniemi (1996), Kiviniemi and Telenius (1998), Heinken et al. (2001) and Heinken and Raudnitschka (2002). Long and curly furs seem generally better suited for seed dispersal than short furs (e.g. sheep versus goat, Shmida & Ellner 1983), yet detailed comparative studies of fur impact on epizoochory are generally lacking. Agnew & Flux (1970) suggested that the sex of an animal might influence seed dispersal through behavioural differences. Animal behaviour such as lying, wallowing and rubbing should allow the picking up of seeds of small-sized plants or from the soil (Fischer et al. 1996), and can influence seed deposition (e.g. wild boars lose many seeds under rubbing trees, Mrotzek et al. 1999). Depending on the animal species and its home range, seeds can be dispersed over varying distances. Experimental studies with marked seeds suggest that small mammals such as mice can disperse seeds over distances in the order of magnitude of metres (Kiviniemi & Telenius 1998), while for larger herbivores such as roe deer and cattle, dispersal distances of tens of metres to a kilometre are proposed (Kiviniemi 1996). Fischer et al. (1996) even reported retention times of weeks to months in the fur of sheep, suggesting dispersal distances of up to 100 km by a migrating flock of sheep.

Endozoochory

Although endozoochory also includes the consumption of fleshy fruits by frugivores (Debussche and Isenmann 1989, Amico and Aizen 2000, Tabarelli and Peres 2002), we focus on the consumption of non-fleshy seeds of mostly grasses and herbs by herbivorous mammals (Janzen 1984, Welch 1985, Malo and Suárez 1995a, Pakeman et al. 1998, Heinken et al. 2001, Cosyns 2004, Cosyns et al. in press a, b). Also carnivores can disperse seeds in their gut, as they feed on herbivores and supplement their diet with fruits (Chavezramirez & Slack 1993). Edible vegetative plant parts ('foliage is the fruit' theory, Janzen 1984) and seed resistance to digestion (Pakeman et al. 2002) are hypothesized to be adaptations to endozoochory by large herbivores. Recent studies reported that far more plant species than previously thought are dispersed by endozoochory, including many species traditionally classified as 'unspecialized', or adapted to other dispersal modes (see Pakeman et al. 1998, Pakeman 2001, Cosyns et al. in press a). Experimental studies demonstrated the high cost imposed by endozoochorous dispersal. The survival percentage of most ingested seeds is relatively low, and decreases further if the passage rate through the herbivore gut is low (Gardener et al. 1993, Cosyns et al. in press b). Although endozoochory is supposed to mediate the dispersal of larger quantities of seeds (Janzen 1984), statements on the relative importance of epi- and endozoochory remain highly speculative. Because of the mechanistic differences between both processes, most studies treat only one of these phenomena (but see Fischer et al. 1996, Stender et al. 1997, Heinken et al. 2002). Hence, the relationship between epi- and endozoochory has been largely neglected in the literature.

Animals and directed dispersal

Besides mere dispersal, animals may provide a means of ‘directed dispersal’ (Janzen 1984, Wenny 2001, Ernst et al. 1992) e.g. for many species relying on disturbed habitats, which are frequented and even generated by animals. By creating gaps in the vegetation during feeding, fighting, wallowing, trampling or litter removal, herbivores provide germination sites for seeds, and contribute as such to the establishment of plants. Naiman (1988) also stressed the ecological impact of animals on plant community structure and biogeochemical cycles, through physical disturbance and feeding strategies.

Seed dispersal in relation to the history of the western European landscape

Several major changes have marked the western European landscape throughout the history of human settlement. The evolution in land use and the progressive fragmentation of (semi)natural habitats (e.g. forests, Tack et al. 1993; heathlands, Burny 1999, Piessens et al. 2004; grasslands, Mitlacher et al. 2002) have severely impacted on plant seed dispersal, mainly through a collapse of the available seed pool in the landscape, in combination with an increasing functional isolation of habitat patches. In addition, human constructions such as road networks present physical barriers to the dispersal of organisms (Lode 2000, Kramer-Schadt et al. 2004).

There has also been a continuous evolution in prevailing seed dispersal mechanisms in our landscapes. Whereas wind, water and wild animals were the main dispersal vectors before human settlement, additional dispersal modes appeared with man (Poschlod & Bonn 1998). Traditional agricultural practices, including livestock and crop breeding, assured a continuous flow of seeds through the different functional parts of the agricultural landscape (arable fields, road margins, pastures, coppiced woods, heathland, peatland, ditches, ...), both through livestock-mediated seed dispersal and through human activities (haymaking, manuring, sod-cutting, artificial flooding, ...). The industrial revolution preceded a number of (agricultural) developments associated with changing dispersal accents. The development of agricultural machinery and the discovery of artificial fertilizers led to the loss of many dispersal connections between different land use segments. On the other hand, the machinery itself functioned as dispersal vector (Strykstra 1996, 1997; Couvreur & Hermy 2002). Natural dispersal mechanisms generally declined, e.g. natural flooding became restricted and populations of most wild animals decreased as a result of hunting and resource competition (e.g. Jedrzejewska et al. 1996, Halley & Rosell 2002) -although some species ultimately expanded as a result of human activities, e.g. fox and roe deer (Tack et al. 1993). The mobility of livestock became drastically restricted on a local scale (stable management versus free-ranging and transhumance), but increased on a regional scale as a consequence of motorized transports. Through trade and traffic -e.g. in the mud of car wheels (Zwaenepoel & Hermy 1998), in air currents of trains (Tikka et al. 2001), through material transport, wool import and garden escapes (Hodkinson & Thompson 1997)- modern society has facilitated the long-distance dispersal of many plants. Numerous human-mediated invasions of plant communities by exotic species have been reported (Vitousek et al. 1997), many of which threatening local biodiversity. In general, the landscape-scale connections between habitats have become smaller, whereas the regional and global connectivity has increased explosively, especially in the last decades (Forman 1998).

Seed dispersal and nature conservation

In the last decades, the growing awareness of the devastating effects of environmental pollution and habitat destruction on global and local biodiversity (Dirzo & Raven 2003) triggered an increasing number of investments in local and global nature conservation. In our regions, most remaining species-rich habitats are semi-natural vegetation types, where centuries of human land use, in combination with natural processes, have resulted in communities of spontaneous, wild, native plant and animal species. To conserve these habitats, a wide range of nature management measures are currently applied, aiming at providing the necessary processes to maintain local species-richness. Such interventions can include ‘external management’, e.g. the restoration of the hydrological conditions of an ecosystem, as well as ‘internal management’, such as mowing, grazing and cutting (Hermy et al. 2004).

One of the important problems in nature conservation and restoration, however, relates to the seed dispersal bottleneck. In our highly fragmented landscape, where the connections (habitat-continua) between semi-natural habitats have been drastically reduced, seed availability is becoming a bottleneck for long-term plant survival (Turnbull et al. 2000, Verheyen et al. 2003a, b). Hence, current efforts to preserve or restore the biodiversity and characteristic species assemblages of semi-natural vegetations are frequently confronted with seed dispersal limitation (Strykstra 2000, Van Groenendael et al. 1998, Bakker & Berendse

1999, Pywell et al. 2002). This has serious implications, as global change (especially climate change) compels plants to migrate through the landscape. The lack of connections between habitats will therefore lead to severe future losses of plant species and communities. Even without further habitat change future species loss may be expected, because of the time lag (50-100 years) in the response of plant species diversity to changing habitat configurations (i.e. ‘the extinction debt’, cf. Lindborg & Eriksson 2004).

Nature management measures impacting on seed dispersal thus deserve the attention of researchers. Active reintroduction of species (Van Groenendael et al. 1998, Strykstra 2000) is generally seen as a last resort to save species from extinction, when habitat amelioration and restoration of dispersal vectors have failed (Van Groenendael et al. 1998). Regarding the restoration of dispersal vectors, much remains to be explored, certainly with respect to the role of zoochory.

Given the principal and historical role of large herbivores (wild and domesticated) in the dynamics of our landscapes (Poschlod & Bonn, 1998), it is not surprising that current nature conservation directives recognize and aim at restoring the keystone role of large herbivores in ecosystem dynamics (see Lundberg & Moberg 2003). Domesticated grazers are therefore introduced at low densities in many nature management and restoration projects. In Flanders, the number of grazed nature reserves is increasing rapidly (Fig 1.4). Because of the limited food supply in the generally small reserves, motorized transports of the herbivores between nature reserves are common practice. The introduced large herbivores are expected to enhance the variation in soil compaction, nutrient availability (Archer 1973, Edwards & Hollis 1982), vegetation composition (Bakker 1998, Bokdam & Gleichman 2000, Bullock & Armstrong 2000), vegetation structure (Olff et al. 1999) and forage quality, as a consequence of selective habitat use (Lamoot et al. 2005), which includes feeding, trampling and defecating (Cosyns & Hoffmann 2004). However, the herbivores may also significantly contribute to (long-distance) seed dispersal, another function formerly provided by our (extinct) wild animals and the free-ranging and migrating herds of domestic livestock (Pykälä 2000). Moreover, as a result of the current transports of herbivores used in nature management, seed dispersal connections between isolated nature reserves at long distances are established. Large herbivores may thus provide potentially important seed dispersal services (e.g. Gibson et al. 1987), which should be further investigated in order to allow application in nature management in general.

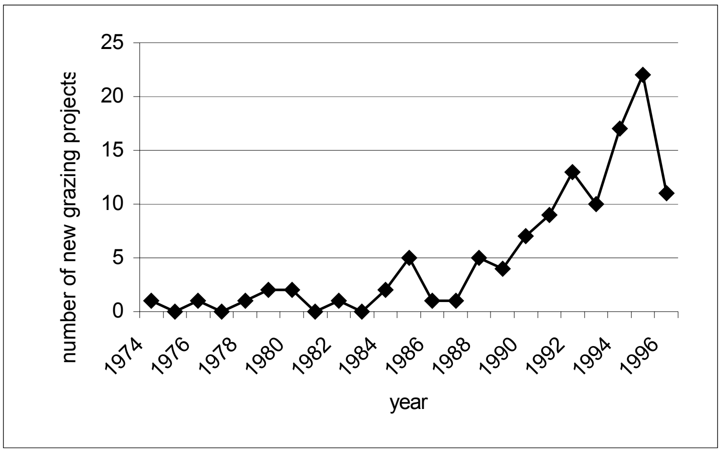

Fig 1.4 *The number of nature reserves in Flanders where new grazing projects with large herbivores were initiated (after Eggermont et al. 1996).*

Investigating zoochorous seed dispersal

As outlined above, (potential) long-distance dispersal mechanisms such as zoochory are key processes in past, present and future plant ecology. However, more data on the mechanics and outcome of these mechanisms (especially on the tail of the dispersal curve) must be collected (Cain et al. 2000) to make informed predictions on the likely outcome of habitat fragmentation, global warming and nature management decisions.

The quantification of long-distance dispersal is extremely challenging (Cain et al. 2000, Levin et al. 2003, Nathan et al. 2003). Genetic methods are rarely applied on seeds (Cain et al. 2000, but see Godoy & Jordano 2001). Unconventional methods that focus on individual movements and methods that couple modelling and empirical tools are put forward as the most promising ways to estimate long-distance dispersal (see Greene & Calogeropoulos 2002, Nathan et al. 2003, Wang & Smith 2002). As most long-distance dispersal vectors are difficult to follow, the availability of suitable and large datasets is limited. In the context of zoochory, seed dispersal can be directly observed if animals carrying seeds in their fur or gut could be followed from the place where the seeds are picked up or ingested to the place where they are released (cf. Wenny 2000). As this is often not feasible, also other approaches should be adopted to unravel the details of the dispersal process. Experimental set-ups based on the controlled attachment of seeds to animal fur (Sorensen 1986, Fischer et al. 1996, Kiviniemi

1996) or the feeding of experimental seeds to animals (Gardener et al. 1993, Cosyns et al. in press b) can offer insight in the factors influencing zoochorous retention times. In combination with details on plant characteristics and animal behaviour, such data can be integrated in mechanistic models for predicting exact seed distributions. Besides parameters describing the dispersal process, such models also require quantification of detailed behavioural information. Further development of mechanistic dispersal models, especially of seed dispersal by animals, has been identified as an important direction for future research (Levin et al. 2003). Therefore, a solid knowledge of the natural history of the dispersal process is required, including high-quality data for parameter estimation and for testing model predictions.

For the investigation of zoochory, wild animals and/or domesticated animals can be studied. Both have (had) an impact on the development of our natural and semi-natural ecosystems (e.g. calcareous grasslands have evolved under sheep grazing, Poschlod et al. 1998), hence both are worth examining with respect to seed dispersal. To asses the potential zoochorous role of our extinct wild large herbivores, *Bos taurus* (van Vuure 2003) and *Equus ferus* (Jordana et al. 1995), we have to rely on studying their domesticated progeny. For this purpose, breeds in which many of the original 'primitive' characteristics relevant to seed dispersal have been conserved are preferable to breeds which have lost these. The variation between a summer and a winter coat (van Vuure 2003) is such an relevant characteristic, which is maintained in many robust herbivore breeds. Galloway cattle, Scottish highland cattle, Heck cattle, Aberdeen Angus cattle, Konik horse, Iceland horse, Exmoor horse, Fjord horse, Haflinger horse and Shetland horse are robust breeds which are frequently used for grazing projects in European nature reserves. Among these animals, considerable variations in fur type occur (e.g. long-furred Galloway cattle versus short-furred Aberdeen Angus cattle). It seems therefore advisable to take this variation into account in epizoochory research, because detailed knowledge about the appearance of the extinct large herbivores is lacking (but see van Vuure 2003). Hence, the use of (various) breeds of robust large herbivores may not only provide useful information about zoochory by domesticated large herbivores, but is probably also the best way to assess the extinct wild herbivores' potential role in zoochory. Moreover, it might also reveal important knowledge about the mechanistics of seed dispersal in general, which may also apply to other wild mammals which are more difficult to examine.

Objectives and outline of the thesis

In spite of the crucial importance of long-distance dispersal (especially for species which cannot disperse in time through the formation of persistent seed banks, see Fig 1.1) and despite the qualitative identification of important long-distance dispersal vectors (e.g. animals), our knowledge of these mechanisms is still very limited and highly fragmented. Zoochory is still generally considered a neglected aspect of dispersal ecology (Levin et al. 2003). Compared to endozoochory, epizoochory has received even less attention, as the fate of epizoochorously dispersed seeds is even more difficult to study (Pakeman 2001). To allow a better and more reliable understanding of epizoochory, ecologists should examine a larger number of animals than previously done (Greene & Calogeropoulos 2002), both for statistical reasons and to allow a better assessment of the range of species susceptible to epizoochory. In addition, they should experimentally quantify the details of epizoochory, and finally construct (simulation) models synthesizing the dispersal process.

Hence, in this study, we aim at providing additional knowledge about (epi)zoochory (see also Fig. 1.3), and at creating a backbone for integration of field data into a model. Our principal objectives are:

1. To collect empirical data on epizoochory on a large number of animals;
2. To compare epi- and endozoochory with respect to species composition and plant traits;
3. To experimentally quantify the capacity of different seeds to adhere to animal fur;
4. To measure seed dispersal distances in field conditions;
5. To integrate experimental and observational data into a simulation model of epizoochory, which accounts for complex animal behaviour, and which allows the prediction of realized epizoochorous dispersal distances (seed shadows) and numbers of seeds dispersed in animal fur.

To reach these objectives, we mainly focus on large herbivores used for grazing in nature reserves, as they (1) can serve as models for (wild) mammals in the present, past and future, (2) are relatively easy to examine, and (3) play a major role in contemporary nature management in our regions (Eggermont et al. 1996, Cosyns & Hoffmann 2004).

As depicted in the schematic overview in Fig. 1.5, this study contains two descriptive, two experimental and one modelling chapter.

Descriptive part

Chapter 2

Grazing by introduced large herbivores has become a wide-spread nature management measure in Flanders. As most Flemish nature reserves are small, the herbivores are regularly transported. As such, they may disperse seeds within and between these nature reserves. In this chapter, a descriptive study of epizoochory in the context of nature management in Flanders is presented. The epizoochorous seed loads of a large number of herbivores (Galloway cattle, horses, donkeys) is examined in terms of species composition, seed number and plant traits, and the ‘mobile link’ function of large herbivores for seed dispersal is discussed.

Chapter 3

Epi- and endozoochory are two important long-distance dispersal mechanisms, yet their relative importance and relationship have hardly been studied. In this chapter, we compare both mechanisms, focusing on large herbivores in a coastal dune nature reserve. We characterize the epi- and endozoochorous species composition in terms of species traits and dispersal-relevant plant types, in order to reveal a possible additive and/or complementary role of both dispersal mechanisms. As large domestic herbivores can be considered as models for (wild) animals in the present, past and future, we put our results in a general ecological context as well as in a nature management context.

Experimental part

Chapter 4

Seeds with morphological adaptations facilitating adhesion to animal fur (e.g. hooks, bristles) are traditionally classified as being epizoochorously dispersed. However, recent studies suggest that such adaptations are no prerequisite for adhesive dispersal, and that in theory almost all species may occasionally be dispersed by epizoochory. In this chapter, we present a standardized method to define the adhesivity of seeds to animal fur in laboratory conditions. We use a large set of seed species and animal furs, to allow the ranking of seed species in terms of adhesivity to different furs. Seed and fur characteristics are used to explain the observed differences in seed adhesivity among seeds and furs.

Chapter 5

Little is known about retention times of seeds in animal fur, and, hence, about potential dispersal distances of the seeds. In this chapter, we describe a field experiment in which marked seeds are attached to the fur of two large herbivore species. We investigate the role of seed morphology, animal fur, vegetation structure and weather condition on the retention time and potential dispersal distance of the seeds.

Modelling part

Chapter 6

Dispersal modelling is receiving increased attention, yet few models incorporate long-distance seed dispersal. The development of models of zoochory is generally restricted by the lack of data for the distillation of model parameters and for model testing. In this chapter, descriptive and experimental data are integrated in a spatially explicit simulation model. Based on the experimental results of chapters 4 and 5, and on additional data of animal behaviour and local plant abundance, parameters are derived to simulate the epizoochorous dispersal of selected plant species. The results of the observational study in chapter 3 are used as an independent source of data for validation of the model, which is used to predict seed shadows and seed numbers in animal fur.

Concluding remarks

Chapter 7

In the last chapter, the results of the previous chapters are briefly summarized, and discussed with respect to methodological limitations and further research. Finally, the consequences for nature management are adressed.

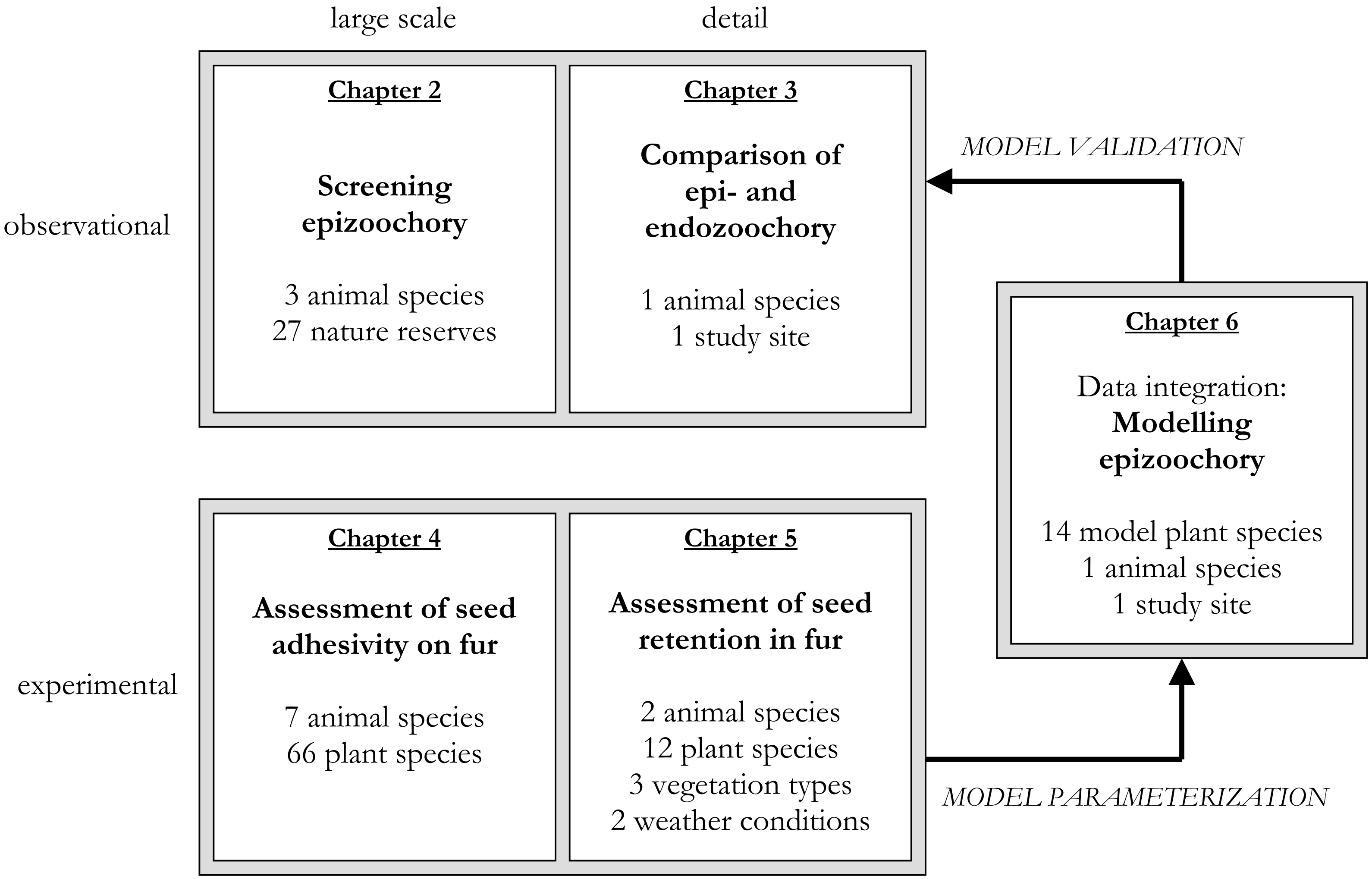

Fig. 1.5 *Schematic overview of the thesis.*

Chapter 2

Large herbivores as mobile links within and between isolated nature reserves through adhesive seed dispersal

Abstract

Dispersal of plant seeds in fur of mammals (epizoochory) is considered an important long-distance dispersal mechanism in present and former plant migration. However, few large datasets based on field observations exist. In this paper, epizoochory was studied by collecting 201 epizoochorous samples from fur of Galloway cattle, donkeys and horses in 27 nature reserves. The seasonal grazing system in Flanders, which involves regular transport of herbivores between nature reserves, allowed detection of seed dispersal both within and between reserves. In total, 6385 seedlings of 75 species germinated from the samples, yet the real seed quantities were underestimated through the seedling emergence method. A variety of seed morphologies, seed weights and plant heights was represented among the dispersed species, 45% of which had a transient seed bank. There was also a gradual turnover in epizoochorous species composition in the course of the vegetation season. We conclude that large herbivores, being models for (wild) mammals in the present and the past, are important dispersers of many plant species. Through seasonal grazing, domesticated herbivores function as ‘mobile link organisms’ connecting isolated nature reserves through seed dispersal, hereby possibly influencing vegetation development and long-term survival of plant populations. Therefore, large herbivores can be important instruments in ecological restoration, especially in fragmented ecosystems.

Keywords

Epizoochory, cattle, donkey, grazing, horse, long-distance seed dispersal, nature management, plant traits

Introduction

Long-distance plant dispersal has recently received much attention, mainly because of its importance for migration in a rapidly changing landscape. The fitness of isolated plant populations depends on successful dispersal between populations, as do the creation and maintenance of a metapopulation (Eriksson 1996, Cain et al. 2000) and the necessary species migrations in response to environmental changes (Watkinson & Gill 2002).

In postglacial times, wild animals were probably key long-distance dispersal vectors, through external (epizoochory) and internal (endozoochory) seed dispersal (Poschlod & Bonn 1998, Pakeman 2001). Nowadays, however, their movement and migration patterns are dramatically restricted, particularly in the present western European fragmented landscape. In semi-natural systems, most dispersal processes have disappeared with the abandonment of traditional agricultural techniques (Poschlod & Bonn 1998, Bruun & Fritsbøger 2002), e.g. transhumant sheep shepherding in calcareous grasslands (Fischer et al. 1996, Poschlod et al. 1998). Long-distance seed dispersal has now become a bottleneck for vegetation development and restoration of isolated (semi-)natural relicts (e.g. Strykstra et al. 1998a, Pywell et al. 2002), particularly for species with short-lived seed banks. Detection of long-distance dispersal in the present-day landscape, which is subject to climate change, habitat destruction and fragmentation, is therefore crucial (Cain et al. 1998, Cain et al. 2000, Pakeman 2001). More data on long-distance dispersal are also indispensable for improving present models, which fail to explain observed plant migration rates (Higgins & Richardson 1999, Greene & Calogeropoulos 2002, Levin et al. 2003). Tracing long-distance dispersal, however, is intrinsically difficult. Because genetic approaches (Cain et al. 2000) are in practice only feasible for detecting seed movement if it results in successful recruitment (Nathan & Muller-Landau 2000), direct field observations of long-distance seed dispersal remain invaluable for understanding plant migration.

Epizoochory is one of the most important long-distance dispersal mechanisms. It has recently been documented by Fischer et al. (1996), Kiviniemi (1996), Stender et al. (1997), Kiviniemi & Telenius (1998), Kiviniemi & Eriksson (1999), Heinken (2000), Heinken & Raudnitschka (2002), Graae (2002). Still, the need for more data on occasional long-distance dispersal events has been stressed (Levin et al. 2003), especially with respect to the number of animals, to be followed for larger distances than anyone has done so far (Greene & Calogeropoulos 2002).

In this study, we examine epizoochory on a large number of herbivores and we detect long-distance seed movement, although human-induced. We focus on domesticated large herbivores, grazing in nature reserves in Flanders, Belgium. Grazing is a major management measure to maintain the semi-natural vegetation types in these reserves. Domesticated herbivores are therefore introduced at low densities, to continue the processes that shaped the semi-natural vegetation during centuries of traditional-agricultural land use. Several species, mostly cattle (often Galloway cattle), horses and sheep, but also donkeys and goats, do currently graze in Flemish reserves. Due to the small area and therefore limited food supply of most reserves, parts of the herds are regularly transported to other nature reserves. Many herbivores spend the winter on a central farm and return to the reserves in spring. These 'nomadic' animals sometimes travel the whole area of Flanders in their life span. Anecdotal observations of seeds in the fur of these transported herbivores suggested that they function as 'mobile link organisms' (see Lundberg & Moberg 2003), providing not only seed transport within individual nature reserves, but also seed exchange between suitable habitats at long distances. Therefore, we examined the epizoochorous seed loads of Galloway cattle, donkeys and horses in actual nature management situations in Flanders. The following questions were addressed:

- What are the species and quantity of seeds dispersed epizoochorously by large herbivores?
- Which dispersal-relevant plant traits are represented among the epizoochorously dispersed seeds?
- Do the three herbivore species differ in their epizoochorous dispersal capacity?
- Does the epizoochorous species composition change during the vegetation season? Finally, the consequences for nature conservation and management are discussed.

Botanical nomenclature follows Lambinon et al. (1998).

Material and methods

Study areas

Studies were carried out in 27 nature reserves in Flanders, Belgium (Fig. 2.1, App. 2.1), ranging in size from a few to more than 300 ha. The reserves are isolated relicts of former species-rich, semi-natural landscapes, which are now heavily fragmented as a result of land use changes. The vegetation consists mainly of species-rich grasslands, sometimes interspersed with areas of tall herbage or woody vegetations.

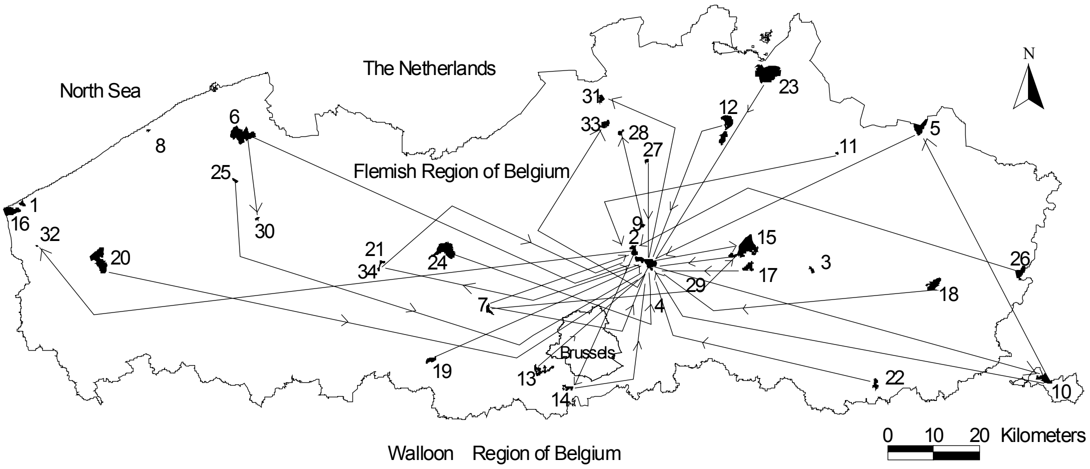

Fig. 2.1 *Flemish nature reserves where epizoochory by large herbivores was investigated. The arrows represent transport routes for seasonal grazing of part of the sampled herbivores. In all reserves except 1, 3, 8, 16, 19 ('no transport'), at least part of the samples were collected after transport of the herbivores from a 'donor' reserve (2, 4-7, 9-10, 11-15, 17-18, 20-27) to a 'receptor' reserve (2, 4, 5, 10, 14-15, 28-34) (see App.2.1 for reserve names).*

Sampling method

Between May 2000 and February 2001, epizoochorous seeds were collected from 201 large herbivores: 125 Galloway cattle from 23 different reserves, 30 horses (Konik, Haflinger and Iceland breeds) from six reserves and 46 donkeys from three reserves (see App. 2.1). These numbers roughly reflect the ratio between the different herbivore species in Flemish nature reserves. The epizoochorous seeds were collected by brushing the fur of each animal for 15 minutes with a very fine horse brush. For practical reasons, the sampling mostly coincided with capture events for veterinary control or transport of animals. Although this resulted in an ‘unbalanced’ sampling, it allowed us to intercept epizoochorous seeds which would actually be displaced over long distances. Of the 201 sampled animals, 82 were brushed after transport from a ‘donor’ reserve to a ‘receptor’ reserve (Fig. 2.1, App. 2.1). This allowed us to witness and quantify seed dispersal between different nature reserves.

Seed germination

After removal of the excess of hair, the samples were sown in trays with sterilized potting soil (seedling emergence method, cf. Ter Heerdt et al. 1996). The trays were kept in laboratory conditions, with a daily light-exposure of 16 hours, daily watering and occasional soil disturbance to expose buried seeds to light. The easily recognizable seeds of seven taxa (see Fig. 2.2) were counted before being sown, to estimate their germination success. Over a period of six months, seedling numbers and species were recorded, and removed to prevent competition. Unrecognizable seedlings were transplanted to separate pots until identification was possible. So that germination of very short-lived seeds was not compromised, a cold treatment of 2 months at 4°C (to imbibed samples) was only given after six months, when germination had ceased. Subsequently, germination was followed up for another three months.

Data analysis

Given the nature of the data, non-parametric statistics were used and relevant data were selected according to the questions posed.

Firstly, the germination success of seven taxa was evaluated by comparing the number of sown seeds with the number of seedlings germinated during the pre- and post-chilling germination cycle.

Secondly, to assess the variety of some dispersal-relevant plant traits represented among the epizoochorous species, we recorded their ‘dispersal agency’ category and the rank of plant height and seed weight (Grime et al. 1988). A Kruskal Wallis test (Siegel & Castellan 1988) was used to test whether the mean seed weight was higher in certain dispersal-agency categories. Since species with short-lived seed banks depend on dispersal in space more than species which can also disperse in time through seed bank formation, we calculated each species’ seed bank longevity index. This is the ratio of the number of records of persistence in soil to the total number of records (transient + persistent) in seed banks (Thompson et al. 1997, Bekker et al. 1998).

Thirdly, the quantity of species and seedlings in the epizoochorous samples was compared between the herbivore species. However, a comparison between herbivores sampled in different reserves or different months was considered to be biased by vegetation variations. Therefore, we limited the statistical comparison (Mann-Whitney U-test; Siegel & Castellan 1988) to the data from ‘Hageven’, the reserve from which most simultaneously collected samples from Galloways and horses were available. Data on donkeys were insufficient to include them in the statistical comparison.

Fourthly, the species composition of the samples was screened for a temporal gradient. To obtain balanced datasets with respect to the number of samples and different sampling months, we selected five reserves with at least 10 non-empty samples collected in at least three different sampling months (Table 2.2). For each of these reserves, the epizoochorous samples were ordinated on the basis of their species composition, using detrended correspondence analysis (DCA, Hill & Gauch 1980), after logaritmic transformation, in the program CANOCO 4.0 (ter Braak & Smilauer 1998). To check if the ranking of the epizoochorous samples along the major DCA-axis reflected the moment of sampling, which would suggest a gradual turnover (temporal gradient) in species composition, Spearman rank correlation coefficients (Siegel & Castellan 1988) were calculated between the sample-scores on the first DCA axis and the sampling month (ranked from May 2000 to February 2001, according to the vegetation season).

Finally, the samples from the 82 transported large herbivores were used to assess the scale of long-distance seed movement between different nature reserves, as a consequence of the seasonal grazing system in Flemish nature management.

Results

Species and seedlings

A total of 6385 seedlings of 75 plant species germinated in the epizoochorous samples of all herbivores (Table 2.1, Fig. 2.3). The chilling treatment enhanced germination of 29 species (21% of the seedlings). For the seven seed species which were counted before being sown, the overall germination success ranged from 6.3% to > 50%, and the post-chilling germination accounted for < 5% to 70% of the germinated seeds (Fig. 2.2). Among the species, 23 plant families were represented, *Asteraceae* and *Poaceae* containing most species (both 24%), and 14% respectively 42% of the seedlings. Except for *Alnus glutinosa* and *Salix cinerea*, all identified species were herbaceous. Of all seedlings, 67% belonged to only five species and 90% to 10 species (Table 2.1).

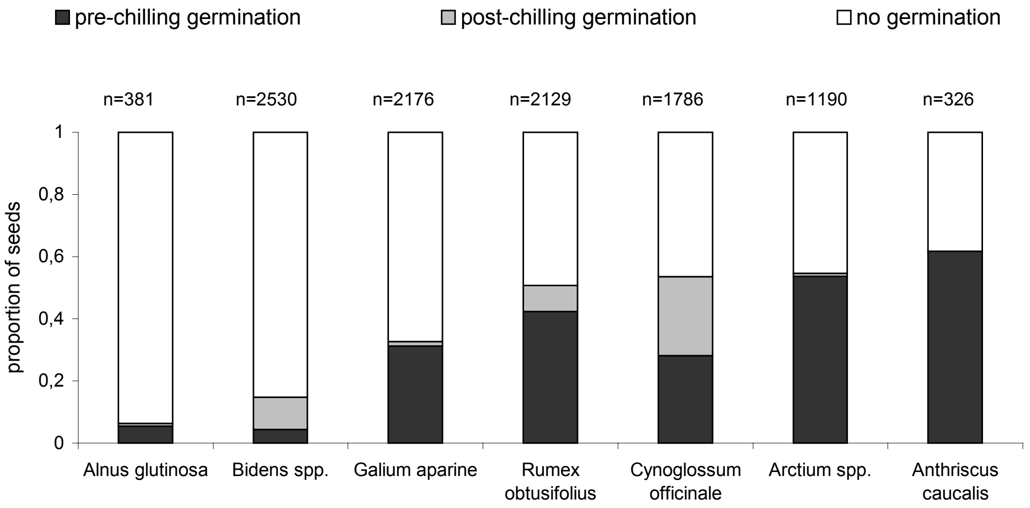

Fig. 2.2 *Germination success of seven species in the pre- and post-chilling germination cycle (n= total number of sown seeds).*

Table 2.1 *Plant species identified in the fur of 201 large herbivores (*: in samples of herbivores transported between reserves). Underlined species are Red List species in Flanders (Biesbrouck et al. 2001).*

Columns 1-2: total number resp. percentage of seedlings in all 201 samples/ Col. 3-4: number resp. percentage of seedlings germinated after the chilling treatment/ Col. 5-7: seedling numbers on each herbivore species (the number of samples containing the species is between brackets)/ Col. 8: number of reserves (n=27) with the species present in at least one sample/ Col. 9: number of months (n=10) with the species present in at least one sample/ Col. 10: dispersal agency (Grime et al. 1988): ANIM= adhesively dispersed by animals (a: dispersule with an awn or with spiny calyx teeth; b: adhesive burr; m: adhesive through mucilage secretion); AQUAT= dispersed by water; WIND= dispersed by wind (p: dispersule plumed or wrapped in woolly hairs; c: seeds small and shed from a capsule from above the surrounding vegetation; w: seeds winged or strongly flattened); UNSP= unspecialized disperser (ag: dispersed as a result of agricultural practices)/ Col. 11: diaspore weight: 1: <0.2 mg 2: 0.21-0.5 mg; 3: 0.51-1 mg; 4: 1.01-2 mg; 5: 2.01-10 mg; 6: >10 mg/ Col. 12: plant height: 1: <100 mm; 2: 101-299 mm; 3: 300-599 mm; 4: 600-999 mm; 5: 1-3 m; 6: 3.1-6 m; 7: 6.1-15 m; 8: >15 m; V: variable (the plant traits in columns 10-12 are based on Grime et al. (1988), or Biesbrouck et al. (2001))/ Col. 13: seed bank longevity index (Thompson et al. 1997, Bekker et al. 1998).

	1	2	3	4	5	6	7	8	9	10	11	12	13
	all seedlings		post chilling		galloway	donkey	horse	number of reserves	number of months	agency of dispersal	diaspore weight	plant height	seedbank longevity
	number	%	number	%	(n=125)	(n=46)	(n=30)						
*Agrostis capillaris**	463	7.25	27	5.8	453 (43)	3 (2)	7 (4)	14	9	UNSP	1	2	0.64
*Agrostis stolonifera**	23	0.36	1	4.3	23 (10)	0	0	5	3	UNSP	1	2	0.38
*Alnus glutinosa**	54	0.85	3	5.6	54 (7)	0	0	3	3	AQ/WIw	4	8	0.30
Anthriscus caucalis	211	3.3	0	0	0	211 (14)	0	1	4	ANIMb	4	3	
Anthriscus sylvestris	3	0.05	0	0	0	1 (1)	2 (2)	2	2	UNSP	5	3	0.03
*Arctium spp.**	771	12.08	13	1.7	634 (6)	137 (6)	0	5	3	ANIMb	6	5	
*Arrhenatherum elatius**	35	0.55	0	0	5 (2)	30 (9)	0	3	3	ANIMa	5	5	0.08

	1	2	3	4	5	6	7	8	9	10	11	12	13
	all seedlings		post chilling		galloway	donkey	horse	number of	number of	agency of	diaspore	plant	seedbank
	number	%	number	%	(n=125)	(n=46)	(n=30)	reserves	months	dispersal	weight	height	longevity
Artemisia vulgaris*	9	0.14	0	0	8 (5)	1 (1)	0	5	5	UNSP	1	5	0.75
Bidens frondosa/tripartita*	542	8.49	323	59.6	541 (9)	1 (1)	0	6	6	ANIMb	5	4	0.56
Bromus hordeaceus*	3	0.05	0	0	1 (1)	2 (1)	0	2	2	ANIMa	5	3	0.24
Cardamine pratensis*	1	0.02	0	0	1 (1)	0	0	1	1	WINDw	3	2	0.49
Carex disticha*	2	0.03	0	0	2 (1)	0	0	1	1	UNSP	4	3	0.22
Centaurea nigra	1	0.02	0	0	1 (1)	0	0	1	1	UNSP	5	3	0.05
Cerastium glomeratum*	1	0.02	0	0	1 (1)	0	0	1	1	WINDc	1	2	0.38
Cerastium semidecandrum	4	0.06	0	0	0	4 (3)	0	1	1	WINDc	1	2	
Chaerophyllum temulum*	1	0.02	0	0	1 (1)	0	0	1	1	UNSP	5	4	
Chenopodium album	5	0.08	0	0	5 (3)	0	0	1	1	UNSP	4	5	0.89
Cirsium vulgare*	1	0.02	0	0	1 (1)	0	0	1	1	WINDp	5	5	0.19
Conyza canadensis*	1	0.02	0	0	1 (1)	0	0	1	1	WINDp	1	3	0.83
Crepis capillaris*	1	0.02	0	0	1 (1)	0	0	1	1	WINDp	2	3	0.14
Cynoglossum officinale	957	14.99	454	47.4	0	957 (32)	0	1	3	ANIMb	6	3	
Dactylis glomerata*	7	0.11	0	0	5 (4)	2 (2)	0	6	4	UNSPag	3	3	0.12
Deschampsia cespitosa*	8	0.13	3	37.5	8 (2)	0	0	1	1	ANIMa	2	3	0.25
Digitalis purpurea	2	0.03	2	100	2 (2)	0	0	1	1	WINDc	1	4	0.88
Echinochloa crus-galli	3	0.05	0	0	3 (1)	0	0	1	1	ANIMa	5	4	1.00

	1	2	3	4	5	6	7	8	9	10	11	12	13
	all seedlings		post chilling		galloway	donkey	horse	number of	number of	agency of	diaspore	plant	seedbank
	number	%	number	%	(n=125)	(n=46)	(n=30)	reserves	months	dispersal	weight	height	longevity
*Epilobium spp.**	48	0.75	1	2.1	46 (16)	1 (1)	1 (1)	9	7	WINDp	1	3	
*Eupatorium cannabinum**	11	0.17	7	63.6	7 (3)	4 (2)	0	2	2	WINDp	2	5	0.33
*Festuca pratensis**	2	0.03	0	0	2 (2)	0	0	2	2	ANIMa	4	4	0.04
*Festuca rubra**	8	0.13	0	0	4 (2)	4 (2)	0	2	2	ANIMa	3	2	0.11
*Galinsoga quadriradiata**	2	0.03	1	50	2 (2)	0	0	2	2	WINDp	1	3	
*Galium aparine**	730	11.43	36	4.9	18 (5)	712 (19)	0	6	4	ANIMb	5	5	0.15
Geum urbanum	1	0.02	0	0	0	1 (1)	0	1	1	ANIMa	3	3	0.08
*Glyceria fluitans**	1	0.02	0	0	1 (1)	0	0	1	1	UNSP	4	4	0.54
Gnaphalium uliginosum	3	0.05	0	0	2 (2)	0	1 (1)	1	2	WINDp	1	2	0.91
Hieracium umbellatum	1	0.02	1	100	1 (1)	0	0	1	1	WINDp	2	V	
*Holcus lanatus**	53	0.83	2	3.8	41 (21)	11 (10)	1 (1)	13	8	UNSP	2	3	0.53
Hypericum perforatum	6	0.09	0	0	4 (3)	0	2 (1)	2	2	WINDc	1	3	0.89
*Hypericum tetrapterum**	1	0.02	0	0	1 (1)	0	0	1	1	WINDc	1	3	0.38
*Juncus articulatus**	1	0.02	0	0	1 (1)	0	0	1	1	ANIMm	1	3	0.88
*Juncus bufonius**	15	0.23	0	0	14 (3)	0	1 (1)	2	4	ANIMm	1	1	0.87
*Juncus effusus**	23	0.36	0	0	23 (4)	0	0	2	2	ANIMm	1	5	0.95
*Lolium perenne**	11	0.17	0	0	10 (7)	0	1 (1)	4	4	UNSPag	4	2	0.11
*Lycopus europaeus**	5	0.08	0	0	5 (4)	0	0	3	2	AQ/AN	2	4	

	1	2	3	4	5	6	7	8	9	10	11	12	13
	all seedlings		post chilling		galloway	donkey	horse	number of	number of	agency of	diaspore	plant	seedbank
	number	%	number	%	(n=125)	(n=46)	(n=30)	reserves	months	dispersal	weight	height	longevity
*Lythrum salicaria**	50	0.78	6	12	39 (4)	6 (2)	5 (3)	4	4	AQUAT	1	4	0.21
*Matricaria spp.**	7	0.11	2	28.6	7 (5)	0	0	3	3	UNSP	1	2	0.44
*Mentha aquatica**	1	0.02	0	0	1 (1)	0	0	1	1	AQUAT	1	3	0.39
*Myosotis arvensis**	25	0.39	0	0	1 (1)	24 (11)	0	2	4	ANIMa	2	2	0.72
Myosotis cespitosa	1	0.02	0	0	1 (1)	0	0	1	1	AQUAT	2	2	
Oenothera spp.	70	1.1	38	54.3	0	70 (6)	0	1	1	WINDc	2	4	
Phacelia tanacetifolia	1	0.02	0	0	0	0	1 (1)	1	1			3	
*Phalaris arundinacea**	1	0.02	1	100	1 (1)	0	0	1	1	AQUAT	3	5	0.06
Phleum arenarium	2	0.03	0	0	0	2 (2)	0	1	2	ANIMa	2	2	
Phleum pratense	2	0.03	0	0	0	2 (2)	0	1	2	ANIMa	2	3	0.12
*Plantago lanceolata**	2	0.03	0	0	2 (1)	0	0	1	1	ANIMm	4	2	0.25
*Plantago major**	14	0.22	1	7.1	11 (10)	1 (1)	2 (2)	8	5	ANIMm	2	2	0.77
Poa annua	6	0.09	0	0	4 (3)	2 (2)	0	4	4	UNSPag	2	2	0.89
*Poa pratensis**	18	0.28	1	5.6	15 (6)	2 (2)	1 (1)	8	6	UNSPag	2	2	0.37
*Poa trivialis**	220	3.45	3	1.4	186 (35)	22 (14)	12 (4)	14	9	UNSPag	1	1	0.75
*Polygonum aviculare**	1	0.02	1	100	1 (1)	0	0	1	1	UNSPag	4	2	0.59
*Polygonum mite**	9	0.14	9	100	7 (4)	2 (1)	0	4	3	UNSP	4	2	
Polygonum persicaria	5	0.08	0	0	5 (2)	0	0	1	1	UNSPag	5	4	

	1 all seedlings number	2 %	3 post chilling number	4 %	5 galloway (n=125)	6 donkey (n=46)	7 horse (n=30)	8 number of reserves	9 number of months	10 agency of dispersal	11 diaspore weight	12 plant height	13 seedbank longevity
*Ranunculus repens**	2	0.03	0	0	2 (2)	0	0	2	2	AQ/AN	5	2	0.67
*Rumex acetosa**	1	0.02	0	0	1 (1)	0	0	1	1	WINDw	3	2	0.25
*Rumex obtusifolius**	153	2.4	54	35.3	127 (27)	1 (1)	25 (6)	16	8	ANIMa	4	5	0.61
Salix cinerea	23	0.36	0	0	21 (2)	2 (2)	0	1	1	WINDp	1	7	
*Scrophularia auriculata**	2	0.03	0	0	2 (1)	0	0	1	1	AQ/WIc	1	3	
*Senecio jacobaea**	1288	20.17	340	26.4	1129 (4)	158 (9)	1 (1)	4	3	WINDp	1	4	0.08
*Senecio vulgaris**	11	0.17	6	54.5	7 (3)	0	4 (4)	3	2	WINDp	1	2	0.58
Sonchus asper	1	0.02	0	0	0	1 (1)	0	1	1	WINDp	2	5	0.52
*Sonchus oleraceus**	6	0.09	0	0	3 (3)	2 (2)	1 (1)	5	4	WINDp	2	5	0.76
Tanacetum vulgare	1	0.02	0	0	0	0	1 (1)	1	1	UNSP	1	4	0.11
*Taraxacum spp.**	1	0.02	1	100	1 (1)	0	0	1	1	WINDp	3	3	0.28
*Trifolium pratense**	1	0.02	0	0	1 (1)	0	0	1	1	ANIMa	4	2	0.14
Trifolium repens	4	0.06	0	0	0	0	4 (2)	1	1	ANIMa	3	1	0.33
*Urtica dioica**	425	6.66	23	5.4	184 (33)	104 (18)	137 (3)	16	7	ANIMa	1	5	0.77
total	6385	100	1360		3692	2483	210						

Plant traits

Eight ‘dispersal agency’ categories were distinguished (Table 2.1). 32% of the species (62% of the seedlings) had adaptations for epizoochory (ANIMa, b, m). Most of these species possessed awns or spiny calyx teeth (ANIMa), but most individuals had burrs (ANIMb). Wind dispersal adaptations, mainly plumes or woolly hairs, occurred in 29.4% of the species (23% of the seedlings). Water dispersal was the main strategy of 10.6% of the species (but only < 1% of the seedlings), and 26.6% were unspecialized dispersers (13% of the seedlings). Seed weight ranged from < 0.2 mg to > 10 mg, and was highest in the category ANIMb (Kruskal Wallis test, KW=25.5, df=7, *P*=0.001). Plant height ranged from < 10 cm to > 15 m, with over 30% of the species < 30 cm. The seed bank longevity index, calculation of which was possible for 77% of the species, indicated that 45% of the species and 52% of the seedlings lacked seed bank formation (longevity index <0.35, Strykstra et al. 2002).

Herbivore species

Seedling and species numbers per sample are summarized in Fig. 2.3. In the non-empty samples (81%), generally a low number of seedlings and species germinated, while only a few animals transported many seedlings (maximum 685) and species (maximum 10). At Hageven, in the January samples, the Galloway cattle dispersed more species and seedlings than the horses: mean 16 seedlings; 2.7 species vs. 0.33 seedlings; 0.33 species (Mann-Whitney U, *P*=0.015 and *P*=0.014, respectively).

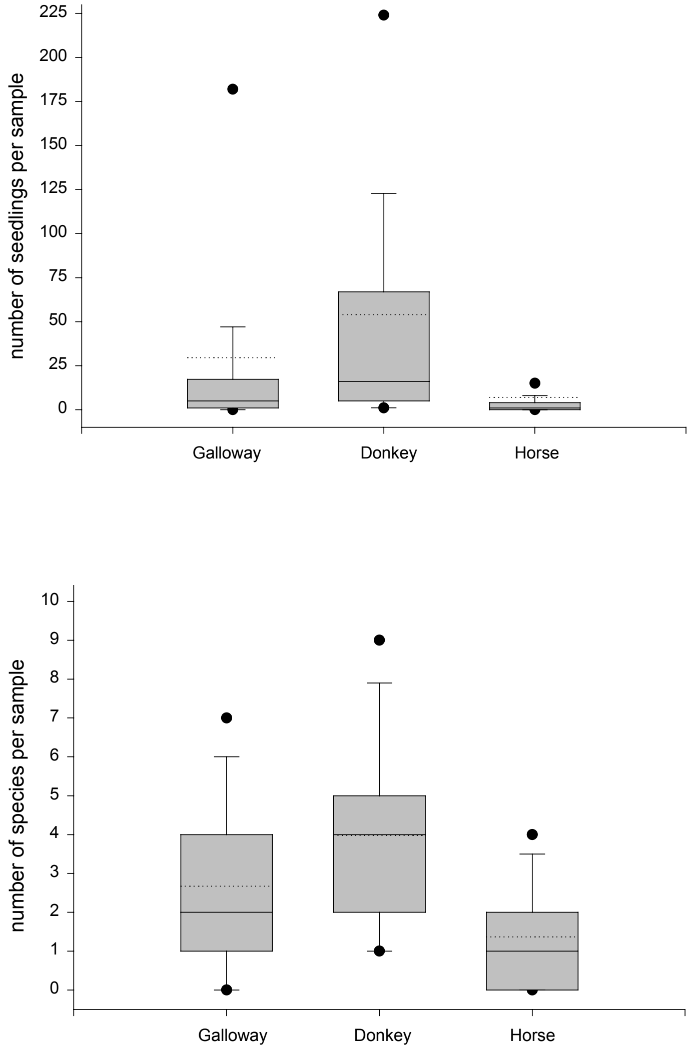

Fig. 2.3 *The number of epizoochorous seedlings (a) and species (b) per sample, for Galloway (n=125), donkey (n=46) and horse (n=30). The full horizontal lines in the grey boxes show the median, the 25th and 75th percentile, and the 10th and 90th percentile. The dashed line represents the mean, and the dots indicate the 5th and 95th percentile.*

Temporal gradient

A temporal gradient in epizoochorous species composition could be detected for three of the five nature reserves where sufficient samples collected in several months were available (Table 2.2). This species turnover was demonstrated by the fact that DCA-ordination of the epizoochorous samples, on the basis of their species composition, resulted in a ranking of the samples according to the month in which they were collected (indicated by a significant correlation between sample-scores on the first DCA-axis and month of sampling).

Table 2.2 *Temporal gradient in epizoochorous species composition, indicated by significant Spearman rank correlations between DCA sample-scores (axis 1) and sampling months (last column), for reserves with at least 10 non-empty samples collected in at least three different months. Month numbers reflect the vegetation season, from May (5) till January (13).*

nature reserve	herbivore species	no. of samples (non-empty)	no. of species	no. of seedlings	sampling months	correlation DCA1 and sampling month
Houtsaegerduinen	D	41	30	2335	6-7-8-10	0.94 ***
Oude Stadswallen	GD	10	15	1384	5-8-11	0.87 **
Mechels Broek	GHD	27	37	1068	5-6-7-9-10-11-13	0.80 ***
Webbekomsbroek	H	13	17	193	8-9-12	0.19ns
Mispeldonk	G	12	17	92	5-6-7-9-11	0.05ns

*G= Galloway ; H= horse; D= donkey; ***P<=0.001; **P <=0.01; ns, not significant*

Long-distance seed movement

The dispersal distances within nature reserves were restricted to the size of the reserves where the herbivores grazed, ranging from 100-200 m to a few km. However, the exchange of large herbivores between different reserves, as part of the nature management system of seasonal grazing, regularly increased these distances significantly. The germination of a total of 2522 seedlings of 52 plant species (Table 2.1) from the epizoochorous material of the 82 herbivores, which were brushed after transport between reserves, revealed the existence of a long-distance seed dispersal pathway between distant and isolated reserves. The travel routes of these seed-carrying herbivores ranged from 2 to 160 km (average 42 km) in our study (Fig. 2.1).

Discussion

This study demonstrates that large herbivores disperse considerable quantities of viable seeds from a wide range of plant species in their fur (201 herbivores transported 6385 seedlings of 75 species; see Table 2.1 and 2.2). Given the number of herbivores used in Flemish nature reserves, the amount of seeds dispersed at a particular moment within or between reserves should be in the order of magnitude of hundreds of thousands. Moreover, the highly variable and relatively low germination performance of some species (Fig. 2.2) suggests that the actual number of dispersed seeds and species is even much higher (see also Stender et al. 1997). It is not clear whether some of the seeds were not viable, or whether germination conditions were suboptimal. However, germination in natural conditions (see Turnbull et al. 2000) is probably much lower than suggested by the seedling emergence method, in spite of the possibilities for ‘directed dispersal’ provided by zoochory (Janzen et al. 1989, Wenny 2001).

Plant traits

A wide variety of dispersal adaptations (adhesive, wind, water, unspecialized) was represented among the epizoochorously dispersed species, but seeds with fleshy fruits (typically dispersed by frugivores) and ant-dispersed seeds were absent from the samples (Table 2.1). Thus, epizoochory is not restricted to seeds with obvious adhesive adaptations, despite the efficiency of these structures (see chapter 4). In combination with the wide range of plant heights (small species can be picked up by lying or wallowing animals; see Fischer et al. 1996) and seed weights (heavy seeds may possess more adhesive properties; see Kiviniemi & Telenius 1998) among the dispersed species, this suggests that most grassland species are, theoretically, capable of adhesive dispersal (see also Fischer et al. 1996).

Herbivore species

In terms of quantity of seeds and species, Galloway cattle seem better dispersers than horses, probably due to their longer hair (see also chapters 4 and 5) and 'bulldozer'-behaviour (cfr. Lamoot et al. 2005). Horses can still be effective seed dispersers over shorter distances (see also chapter 5), as illustrated by the following observation in the 'Webbekomsbroek' reserve: 1080 *Rumex obtusifolius* seeds (from 2129 counted seeds) germinated from three horse samples (not included in the dataset), which were collected after the horses walked through seed bearing *Rumex obtusifolius* vegetation, following a 'normal' sampling (resulting in almost-empty samples). The donkeys were not included in the statistical comparison because they did not share their main home reserve (Houtsaegerduinen) with other large herbivores. The vegetation in this coastal dune reserve was also quite different from most other reserves. This may explain the relatively high numbers of seedlings and species in the donkey samples (Fig. 2.3), including a few very adhesive species (e.g. *Cynoglossum officinale, Galium aparine* and *Anthriscus caucalis*) (see also chapter 3).

Temporal gradient

The epizoochorous species composition gradually changed in time, as demonstrated by the high correlations between the month of collection of the samples and their scores on axis 1 of the ordination plot based on the species composition of the samples (Table 2.2). Such a turnover (see also Fischer et al. 1996; Heinken 2000) results from the fact that seed setting periods vary with species and retention times of seeds in fur are usually relatively short (see also chapters 3 and 5). A temporal gradient in epizoochorous species composition could not be shown for two of the five examined reserves, possibly due to insufficient data (Table 2.2) or to the (accidental) lack of species with a sufficiently delineated seed shedding period in the samples. Several of the abundant epizoochorous species occurred indeed in samples of at least seven different months (e.g. *Agrostis capillaris, Poa trivialis, Urtica dioica, Rumex obtusifolius, Holcus lanatus),* and 25% of all 75 species were observed to be dispersed at least in four different months.

Plant migration, nature management and nature conservation

The examined herbivores serve as models for wild mammals and for herbivores currently used in agriculture and nature management. Similar seed dispersal mechanisms must have occurred in the past, when natural populations of large mammals still populated these regions. Our results may, therefore, help to understand the role of epizoochory in former plant migrations. In the present western-European landscape, most natural populations of large mammals have vanished or have been greatly reduced. Habitat loss and fragmentation have dramatically limited the migration possibilities of animals and hence the distances over which they disperse seeds (Higgins et al. 2003a). We showed that the actual role of domesticated large herbivores may still be considerable in nature reserves, where grazing is an important management measure. Although potential dispersal distances are restricted by the size of the reserves, regular animal transports between reserves (up to 200 km), such as in the seasonal grazing system in Flanders, increase the herbivores' artificial home ranges and thus the seed movement distances (Fig. 2.1). We observed 2522 viable seeds of 52 plant species dispersing between different reserves; since we examined only a fraction of the herbivores transferred annually between Flemish reserves, the actual total seed flow must be considerable. In addition, herbivores also disperse seeds by endozoochory (Janzen 1984, Pakeman 2001, Pakeman et al. 2002, Cosyns 2004), generally with higher seed quantities per herbivore (Cosyns et al. in press a), and a complementary set of plant species (chapter 3). Given the importance of long-distance dispersal (Clark et al. 1998), these animal transfers may be (besides seed dispersal by mowing machinery; Couvreur & Hermy 2002) the only remaining 'link' between isolated nature reserves, in terms of genetically connecting plant populations and of supplying seeds of 'new species', a prerequisite for colonization.

Domesticated large herbivores may, thus, partly adopt the role of (extinct) wild animals in seed dispersal. Consequently, they could be an important instrument in ecological restoration projects, which are often confronted with seed dispersal limitations (Strykstra et al. 1998a, Bakker & Berendse 1999, Pywell et al. 2002). In a restoration context, herbivore movement could be strategically directed in space and time, from target seed sources towards insufficiently developed vegetation. Especially the long-haired Galloway cattle seem suited for this purpose (see also Stender et al. 1997; chapters 4 and 5). Without animals as 'mobile links' (see also Poschlod et al. 1996, Lundberg & Moberg 2003), vegetation development may be much more restricted and slower as a result of dispersal limitation, particularly in new nature reserves established on former agricultural land (see also Briers 2002). The presence in our samples of many species (45%) lacking seed bank formation (in contrast to endozoochory; Pakeman et al. 2002) is also particularly important in a nature restoration point of view, since the loss of species with short-lived seeds can only be counteracted by successful dispersal.

Herbivores as dispersal promoting mobile links could help to counteract the catastrophic effect on biodiversity of the disappearance of free-ranging wild animals and migrating herds (see also Pykälä 2000). Moreover, the herbivores enhance the dispersal opportunities of plants over distances which might be crucial in the face of climate change (see also Watkinson & Gill 2002, Higgins et al. 2003a).

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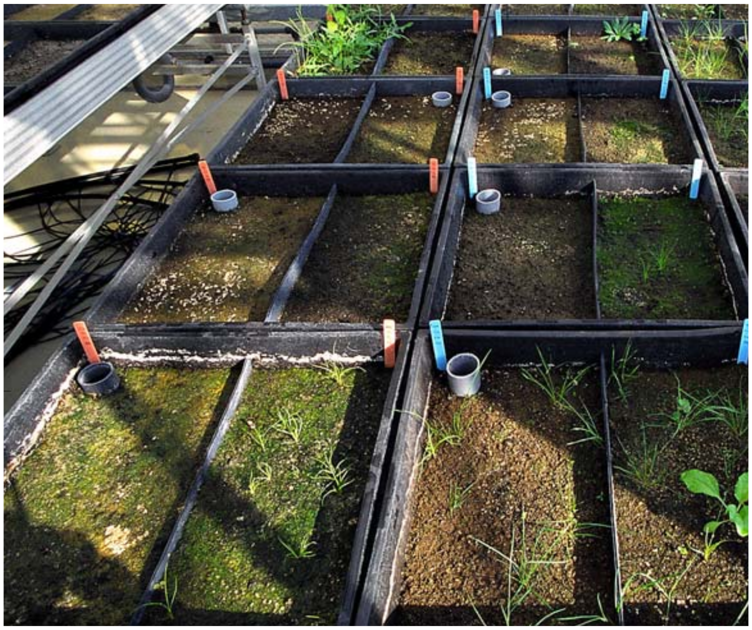
App. 2.1 *List of nature reserves where samples of epizoochorous material were collected (the codes correspond to Fig. 2.1). Columns 3-5 show the sampled herbivore species (G= Galloway ; H= horse; D= donkey), the number of samples (in the case of several herbivore species, numbers are given in the same order as in column 3), and the number of different sampling months. In columns 6-9, the total number of plant species and seedlings identified in the samples of each reserve is given, as well as the mean species and seedling number per sample. In all reserves except those with 'no transport' in column 10, at least part of the samples were collected after transport of the herbivores from a 'donor' reserve to a 'receptor' reserve (transport routes are depicted in Fig. 2.1).*

code	nature reserve	herbivore species	n° of samples	n° of sampling months	total n° of species	total n° of seedlings	mean n° of species per sample	mean n° of seedlings per sample	herbivore transport
1	Houtsaegerduinen	D	41	5	30	2335	4	55.6	no transport
2	Mechels Broek	GDH	24+1+4	7	37	1068	3.2	36.8	donor + receptor
3	Webbekomsbroek	H	18	3	17	193	1.8	10.7	no transport
4	Mispeldonk	G	16	5	17	92	2	5.8	donor + receptor
5	Hageven	GH	10+3	2	9	148	2.2	11.4	donor + receptor
6	Oude stadswallen	GD	8+4	3	15	1384	2.8	115.3	donor
7	Wellemeersen	G	9	3	8	55	1.9	6.1	donor
8	D'heye-Blutsyde	G	8	1	2	14	0.8	1.8	no transport
9	Hondsbossen	G	7	2	10	39	2.7	5.6	donor
10	Altembroek	G	6	3	16	145	6	24.2	donor + receptor
11	Sluismeer	G	4	1	5	30	2.3	7.5	donor
12	Visbeek-Kindernauw	G	4	1	10	28	3.5	7	donor
13	Zuunvallei	G	4	1	4	6	1	1.5	donor
14	Kesterbeekvallei	G	4	1	0	0	0	0	donor + receptor
15	Langdonken	G	4	1	6	93	2.5	23.3	donor + receptor
16	De Westhoek	H	3	1	4	0	0	0	no transport
17	Demerbroeken	G	3	1	0	4	1	1.3	donor
18	Maten	G	3	1	6	12	2.3	4	donor
19	Moenebroek	G	2	1	10	584	7	292	no transport
20	Blankaart	G	2	1	1	1	0.5	0.5	donor
21	Scheldemeersen	G	2	1	12	120	8	60	donor
22	Gelinden	G	1	1	2	2	2	2	donor
23	Hoogmoerheide	G	1	1	0	0	0	0	donor
24	Kalkense Meersen	G	1	1	2	2	2	2	donor
25	Leiemeersen	G	1	1	6	26	6	26	donor
26	Maaswinkel	H	1	1	0	0	0	0	donor
27	Zevenbergen	G	1	1	3	4	3	4	donor
sum			201		75	6385			



Chapter 3

Complementarity of epi- and endozoochory of plant seeds by free-ranging donkeys

Abstract

Epizoochory and endozoochory are well-recognized long-distance seed dispersal mechanisms, yet their relative importance has hardly been studied before. Here, epi- and endozoochory were compared on donkeys foraging in a species-rich 80 ha coastal dune ecosystem, through *in vitro* germination of zoochorous material obtained by fur brushing and dung collection. We identified 6675 seedlings of 66 plant species, covering 20% of the species recorded in the study area. Of the 66 species, only 16 occurred in both epi- and endozoochory samples, demonstrating the complementarity of both dispersal mechanisms. The species composition in the zoochory samples reflected a strong seasonality, and seedling numbers were partly correlated with species abundance in the study area. The non-zoochorously dispersed species in the study area differed from the zoochorous species in seed size and weight, plant height, life span, dispersal strategy and seed bank persistence. Dispersal-relevant plant traits were used to derive dispersal-functional plant types for all species in the study area. Epizoochory showed to be more specific than endozoochory and was associated with a more narrow range of dispersal-functional plant types.

Key words

Coastal dune ecosystem, dung, fur, grazing, large herbivore, long-distance seed dispersal, plant traits

Introduction

Long-distance seed dispersal mechanisms have recently received much attention, especially in the context of understanding and predicting plant migration (e.g. Cain et al. 1998, Clark et al. 1998, Higgins & Richardson 1999, Pakeman 2001). The relevance of seed dispersal research has become increasingly clear given the problem of plant invasions, limited dispersal in fragmented habitats (Poschlod & Bonn 1998, Higgins et al. 2003a), and the necessary plant migrations in response to global change (Watkinson & Gill 2002). Also the insight that seed limitation is a major ecological constraint in several ecosystems (e.g. Zobel et al. 2000, Turnbull et al. 2000, Dalling et al. 2002, Foster & Tilman 2003, Verheyen et al. 2003a, b) has orientated research efforts towards dispersal.

Zoochory, the external and internal dispersal of plant seeds by animals, is an important dispersal mechanism. Epizoochory, on the one hand, is mainly associated with adhesive seed adaptations facilitating attachment to mammalian fur, although also species adapted to other dispersal modes or without obvious adaptations disperse epizoochorously (Fischer et al. 1996, Heinken & Raudnitschka 2002, chapters 2, 4, 5). Endozoochory, on the other hand, covers the consumption of fleshy fruits by frugivores (Debussche & Isenmann 1989, Amico & Aizen 2000, Tabarelli & Peres 2002) and the consumption of seeds of grasses and herbs by herbivorous mammals (Janzen 1984, Welch 1985, Gardener et al. 1993, Malo & Suárez 1995a, Pakeman 1998, Heinken et al. 2001, Cosyns 2004, Cosyns et al. in press a). Edible vegetative plant parts (Janzen 1984) and seed resistance to digestion (Pakeman et al. 2002) are hypothesized to be adaptations to endozoochory by large herbivores.

Both epi- and endozoochory are key factors determining plant distribution (Welch 1985, Malo & Suárez 1995b, Fischer et al. 1996, Bonn & Poschlod 1998, Pakeman et al. 2002). Moreover, they may provide a means of 'directed dispersal' for many species relying on disturbed habitats, which are frequented and even generated by herbivores through feeding, trampling or wallowing (Janzen 1984, Wenny 2001).

Recently, an increasing number of attempts to model zoochorous dispersal has been undertaken (e.g. Pakeman 2001, Higgins et al. 2003a, Vellend et al. 2003). Yet, basic and reliable knowledge of which plants are dispersed zoochorously and which factors and plant traits direct zoochorous dispersal is still very limited. Several authors have therefore explicitly stressed the need for more data on the influencing factors of zoochory (Higgins & Richardson 1999, Bullock et al. 2002, Levin et al. 2003). Studies which fill this gap are scarce, especially comparative studies of epi- and endozoochorous dispersal. Both processes -which are mechanistically very different- have been studied simultaneously on sheep in calcareous grasslands (Fischer et al. 1995), on Galloway cattle in moorland pastures (Stender et al. 1997), and on wild boar and roe deer in a forest ecosystem (Heinken et al. 2002). However, the possible additive and/or complementary effects of epi- and endozoochory, as well as the role of dispersal-functional plant traits in facilitating epi- or endozoochory, remain largely unexplored.

In this paper, we compare epi- and endozoochorous seed dispersal by donkeys grazing in a species-rich coastal dune ecosystem. We test the following research hypotheses: 1) Different plant species are dispersed by epi- and endozoochory. 2) The species abundance in the study area influences the species abundance in the zoochory samples. 3) Morphological and ecological plant traits relevant to dispersal differ between species dispersed by zoochory and species not dispersed by zoochory.

Material and methods

Study area

The study was carried out in the coastal dune nature reserve ‘Houtsaegerduinen’ (80 ha), in the west of Flanders, Belgium (51° 05’ N, 2° 35’ E). The variation in abiotic conditions and the historical land use in this coastal dune system led to a high plant species richness and a wide range of plant communities. Although the dune landscape is dominated by *Hippophae rhamnoides* and *Ligustrum vulgare* shrubs, grassland covers at least one third of the area. Part of the grassland is scattered within the scrub as small and mostly species poor remnants of dune grassland or as species poor *Calamagrostis epigejos* - *Arrhenatherum elatius* dominated patches, which established after scrub degradation. Flowering and fruiting of the plant species is concentrated from April to October. Six donkeys were released in the study area in 1997 for nature management purposes; the herd counted 15 animals at the time of data collection.

Data collection and treatment

The zoochory samples were collected in four different time periods between June and October 2000: early summer (9 June - 19 July), mid-summer (20 July - 19 August), late summer (20 August - 19 September) and early autumn (20 September - 19 October). In the case of epizoochory, 41 samples were collected. Respectively 8, 11, 15 and 7 donkeys were sampled in each of the four time periods, by brushing the entire fur of each individual with a very fine horse brush during 15 min. In the case of endozoochory, the number of sampled donkeys was 4 in early summer, and 8 in each of the three other periods. For practical reasons, the dung of two donkeys was pooled to one sample with a volume of 2.5 L, resulting in 28 sampled donkeys, pooled in 14 samples. Freshly deposited excrements were used, hereby avoiding the lowermost dung part to prevent contamination with seeds of underlying plants or from the soil seed bank.

In the laboratory, the samples were prepared for the seedling emergence method (Roberts 1981, Ter Heerdt 1996). The excess of hair was carefully removed from the dry epizoochory samples, while the endozoochory samples were air-dried in a greenhouse, then stored for two weeks at 4°C (without imbibition of the seeds), and subsequently softly homogenized in a Retsch mill (type SK 100) to allow easy spreading. All samples were sown in trays with sterilized potting soil, and kept in laboratory conditions with a daily light-exposure of 16 h, daily watering and occasional soil disturbance in order to light-expose buried seeds. During six months, species and seedlings were recorded, and immediately removed to prevent competition and flowering. Unrecognizable seedlings were grown separately until identification was possible. After six months, the epizoochory samples were stratified for 2 months at 4°C in imbibed conditions, after which the germination cycle was prolonged for another three months. However, no new species emerged during this second germination period.

The abundance of all plant species in the study area was recorded on several occasions between 1990-2000, using Tansley scaled ordinal abundance estimates for all species in the entire study area, in combination with percentage cover estimates in 1m^2 quadrats. Plant species nomenclature follows Lambinon et al. (1998).

Data analysis

First, to explore overall differences in epi- and endozoochorous species composition, rank-abundance diagrams of the species dispersed by epizoochory and by endozoochory were designed. In addition, the log-transformed dataset of all zoochory samples together was used for Detrended Correspondence Analysis (DCA, Hill & Gauch 1980), using the program CANOCO 4.5 (ter Braak & Smilauer 2003). The sample-scores on the first four DCA-axes were then Spearman rank correlated (Siegel & Castellan 1988) with the date of sampling. The same ordination and correlation procedure was also used for the epizoochory and endozoochory samples separately.

Secondly, the species' abundance in the study area was Spearman rank correlated with the number of seedlings in the samples. This was done for the species in the combined 'zoochory' samples (n=66), the species exclusively in the epizoochory samples (n=13), the species exclusively in the endozoochory samples (n=37), and the species in both epi- and endozoochory samples (n=16), respectively.

Thirdly, morphological and ecological plant traits relevant for dispersal (Table 3.1, 3.3), were compared between a) species exclusively in epizoochory samples, b) species exclusively in endozoochory samples, c) species in both epi- and endozoochory samples, d) species in study area but not in zoochory samples. For this purpose, Kruskal Wallis tests and multiple comparisons (Siegel & Castellan 1988) were used for the continuous and ordinal variables, and Pearson Chi^2 association tests (Siegel & Castellan 1988) for the categorical variables. All traits but plant height, strategy of dispersal and seed bank persistence (derived from Grime et al. 1988) originated from the BIOLFLOR database (Klotz et al. 2002), an elaborate database on biological and ecological traits of vascular plants in Germany.

Fourthly, all species in the study area were grouped into dispersal-functional plant types. These plant types were derived from cluster analysis, based on dispersal-relevant plant traits (Table 3.1, 3.4), using Gower's similarity coefficient and the 'sum of squares' method in the program Clustan Graphics 5.08 (Clustan Ltd. 2001). The emergent clusters were tested for differences in these traits, using Kruskal Wallis tests with multiple comparisons for the continuous and ordinal variables, and Pearson Chi^2 association tests for the categorical ones. The clusters were subsequently named by a dispersal-functional plant type, on the basis of their cluster profiles. Finally, the number of species identified in the samples of (a) exclusively epizoochory, (b) exclusively endozoochory and (c) both epi- and endozoochory, was used to link the dispersal-functional plant types with epi- or endozoochorous dispersal. To check whether the species abundance in the study area, which might influence the probability of zoochorous dispersal, differed between the different clusters, a Kruskal Wallis test with multiple comparisons was used. All statistical analyses were performed using the program SPSS 10 (SPSS 1999), unless mentioned otherwise.

Results

Species composition

From all samples together, 6675 seedlings of 66 plant species germinated, covering 20% of all species in the study area (Table 3.1, App. 3.1). The endozoochory samples contained more species and seedlings, and had a higher diversity than the epizoochory samples (Fig. 3.1). The most abundantly germinating species were *Urtica dioica* (70% of the seedlings in the endozoochory samples), *Cynoglossum officinale* and *Galium aparine* (41% and 31% of the seedlings in the epizoochory samples, respectively). The epizoochory samples contained relatively more grasses than the endozoochory samples (Table 3.1). Of the 66 species, 16 occurred in both epi- and endozoochory samples, 13 were exclusively present in the epizoochory samples and 37 only in the endozoochory samples.

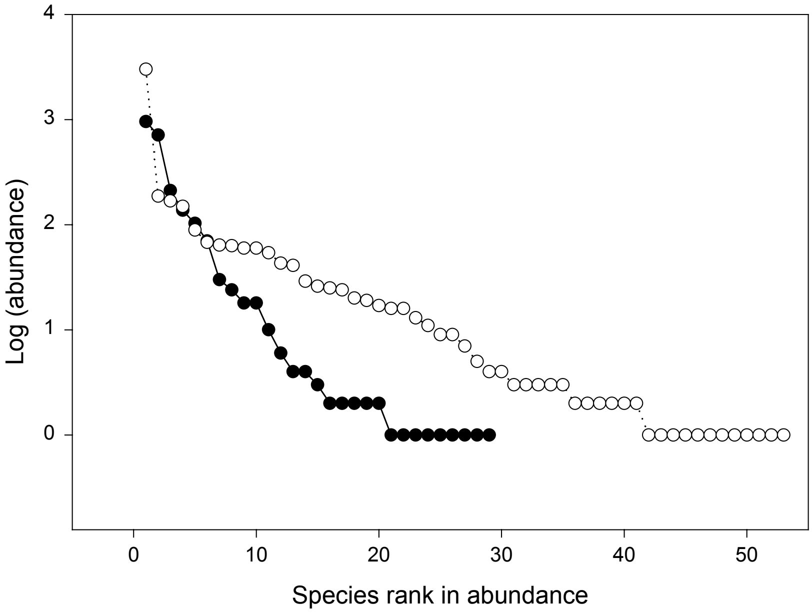


Fig. 3.1 *Rank-abundance diagrams of dispersed plant species by epizoochory (filled symbols) and endozoochory (empty symbols). Species in each group are ranked from commonest (left) to rarest (right).*

Table 3.1 *Overview of the species in the zoochory samples and their relevant traits. Underlined species are Red List species in Flanders (Biesbrouck et al. 2001).*

Species	presence in epi/endo	plants in epi samples	plants in endo samples	number of epi samples	number of endo samples	abundance in study area	seed weight (mg)	seed length (mm)	seed width (mm)	plant height	life span	dispersal strategy	reproduction type	group	seed bank persistence	cluster number dispersal-functional plant type
Achillea millefolium	2	0	4	0	3	3	0.13	2	0.86	2	4	3	2	3	1	4
Agrostis capillaris	12	3	29	2	8	2	0.06	1	0.38	2	4	6	2	1	3	5
Agrostis stolonifera	2	0	41	0	5	2	0.08	1.08	0.45	2	4	6	2	1	3	5
Aira praecox	2	0	3	0	2	2	0.18	2.52	0.48	1	1	1	1	1	2	2
Anthriscus caucalis	12	211	1	14	1	4	1.3	3.77	0.96	3	1	1	1	3	.	2
Arctium minus	1	137	0	6	0	2	7.38	5.73	2.24	5	2	1	1	3	3	2
Arenaria serpyllifolia	2	0	13	0	6	4	0.05	0.6	0.44	1	1	3	1	1	3	3
Arrhenatherum elatius	1	30	0	9	0	5	3.29	3.81	1.22	5	4	1	1	1	1	2
Artemisia vulgaris	12	1	68	1	4	2	0.13	1.46	0.44	5	4	6	1	3	3	5
Bidens tripartita	1	1	0	1	0	1	2.61	6.36	2.18	3	1	1	1	3	.	2
Bromus hordeaceus	1	2	0	1	0	3	3.48	6.08	1.51	3	1	1	1	1	1	2
Calamagrostis epigejos	2	0	26	0	5	5	0.1	1.25	0.4	4	4	3	2	1	.	1
Capsella bursa-pastoris	2	0	1	0	1	3	0.13	0.95	0.47	1	1	3	1	3	3	3
Carex arenaria	2	0	187	0	14	4	0.78	1.83	1.08	2	4	6	3	2	.	5
Cerastium fontanum	2	0	3	0	2	3	0.12	.	.	1	4	6	2	3	3	4
Cerastium semidecandrum	1	4	0	3	0	3	0.04	0.45	0.41	2	1	3	1	3	3	3
Chelidonium majus	2	0	2	0	2	3	0.83	1.36	0.81	3	4	5	1	3	3	3
Chenopodium album	2	0	1	0	1	2	1.5	1.17	1.17	5	1	6	1	3	3	5
Conyza canadensis	2	0	2	0	2	3	0.05	1.21	0.34	3	1	3	1	3	.	3
Crepis capillaris	2	0	2	0	2	4	0.26	2.2	0.53	2	1	3	1	3	3	3
Cynoglossum officinale	1	957	0	32	0	3	26.05	6.78	5.6	3	3	1	1	3	2	2
Dactylis glomerata	1	1	0	1	0	2	0.99	2.77	0.99	3	4	6	1	1	1	2
Epilobium ciliatum	12	1	1	1	1	1	0.07	1.13	0.4	4	4	3	2	3	2	4
Epilobium hirsutum	2	0	2	0	1	2	0.14	0.98	0.44	5	4	3	2	3	3	4
Epilobium montanum	2	0	1	0	1	2	0.12	1.18	0.48	3	4	3	2	3	3	4
Eupatorium cannabinum	2	0	4	0	3	3	0.27	2.97	0.52	5	4	3	2	3	3	4
Festuca rubra	12	4	2	2	2	3	1.3	3.83	1	2	4	6	2	1	1	5
Galium aparine	12	712	60	19	13	4	8.34	3.2	2.63	4	1	1	1	3	1	2
Galium mollugo	2	0	54	0	2	2	0.53	1.22	1.13	3	4	1	1	3	.	2
Galium verum	2	0	60	0	6	4	0.44	1.42	1.2	2	4	6	2	3	1	4
Geranium molle	2	0	5	0	2	3	1.24	1.54	1.09	2	1	6	1	3	2	5
Geum urbanum	1	1	0	1	0	2	2.45	4.54	1.75	3	4	1	1	3	2	2
Holcus lanatus	12	10	89	9	9	4	0.47	2.35	0.84	3	4	6	2	1	3	5
Hypochaeris radicata	2	0	2	0	1	2	.	6	0.75	1	4	3	1	3	2	3
Juncus articulatus	2	0	17	0	7	2	0.02	0.57	0.27	3	4	1	2	2	3	4
Juncus bufonius	2	0	16	0	7	2	0.02	0.39	0.31	1	1	1	1	2	3	2

Species	presence in epi/endo	plants in epi samples	plants in endo samples	number of epi samples	number of endo samples	abundance in study area	seed weight (mg)	seed length (mm)	seed width (mm)	plant height	life span	dispersal strategy	reproduction type	group	seed bank persistence	cluster number dispersal-functional plant type
Koeleria albescens	2	0	3	0	2	3	.	.	.	2	4	6	2	1	.	5
Leontodon saxatilis	2	0	1	0	1	1	.	.	.	2	4	3	2	3	2	4
Lythrum salicaria	12	6	25	2	4	2	0.05	1.03	0.4	4	4	4	1	3	3	3
Myosotis arvensis	1	24	0	11	0	1	0.29	1.43	0.89	2	2	1	1	3	3	2
Oenothera biennis	1	70	0	6	0	3	0.44	1.7	1.25	4	2	3	1	3	.	3
Oenothera glazioviana	2	0	19	0	3	3	0.6	1.51	1.24	4	2	3	1	3	.	3
Phleum arenarium	1	2	0	2	0	3	0.18	1.03	0.6	1	1	1	1	1	.	2
Phleum pratense	12	2	63	2	13	1	1	1.48	0.91	3	4	1	1	1	3	2
Plantago coronopus	2	0	1	0	1	2	.	1.1	0.65	1	3	1	1	3	3	2
Plantago lanceolata	2	0	16	0	4	2	1.81	2.84	1.39	2	4	1	1	3	3	2
Plantago major	12	1	43	1	3	3	0.26	1.5	0.81	2	4	1	1	3	3	2
Poa annua	12	2	24	2	7	3	0.3	1.86	0.7	2	3	6	1	1	3	2
Poa pratensis	12	1	149	1	14	3	0.3	1.6	0.52	3	4	6	2	1	3	5
Poa trivialis	12	18	168	11	12	4	0.17	1.52	0.5	3	4	6	2	1	3	5
Ranunculus repens	2	0	1	0	1	2	1.82	3.09	2.27	2	4	4	2	3	3	4
Rubus caesius	2	0	3	0	3	5	3.61	3.13	1.93	4	4	2	2	4	2	1
Rumex crispus	2	0	1	0	1	2	2.93	2.51	1.58	3	4	6	2	3	3	4
Rumex obtusifolius	1	1	0	1	0	2	2.7	2.29	1.4	3	4	1	1	3	3	2
Sagina procumbens	2	0	1	0	1	3	0.02	0.43	0.27	1	4	6	1	3	3	5
Senecio jacobaea	12	18	9	7	5	4	0.39	2.02	0.5	4	3	3	1	3	3	3
Silene latifolia subsp. alba	2	0	3	0	2	3	0.81	1.35	1.1	4	4	3	1	3	3	3
Solanum nigrum	2	0	1	0	1	2	0.74	1.92	1.36	2	1	2	1	3	.	3
Sonchus asper	1	1	0	1	0	2	0.34	2.7	1.06	3	1	3	1	3	3	3
Sonchus oleraceus	12	2	9	2	6	2	0.53	2.86	0.91	3	1	3	1	3	3	3
Stellaria media	2	0	7	0	5	4	0.48	1.13	1.05	2	1	6	1	3	3	5
Trifolium dubium	2	0	11	0	3	3	0.36	1.25	0.86	2	1	1	1	3	3	2
Trifolium repens	2	0	20	0	3	2	0.59	1.17	1.02	1	4	1	2	3	3	4
Urtica dioica	12	103	3010	17	10	3	0.19	1.19	0.77	4	4	1	2	3	3	4
Veronica chamaedrys	2	0	64	0	10	3	0.22	1.18	1	1	4	6	2	3	3	4
Vicia cracca	2	0	1	0	1	4	14.29	2.89	2.81	4	4	6	1	3	1	5

Presence in epi/endo samples (1, epizoochory samples; 2, endozoochory samples; 12, both epi- and endozoochory samples); Abundance in the study area (1, present; 2, occasional; 3, frequent; 4, codominant; 5, dominant); Plant height (1, <100 mm; 2, 101-299 mm; 3, 300-599 mm; 4, 600-999 mm; 5, 1-3 m; 6, 3.1-6 m; 7, 6.1-15 m; 8, >15 m); Life span (1, annual; 2, biennial; 3, annual/biennial to perennial; 4, perennial); Dispersal strategy (1, adaptations for epizoochory; 2, ingested berries; 3, adaptations for anemochory; 4, adaptations for hydrochory; 5, adaptations for myrmecochory; 6, unspecialized); Reproduction type (1, only by seed; 2, mostly by seed; 3, by seed and vegetative; 4, mainly or exclusively vegetative); Group (1, grass; 2, sedge; 3, herb; 4, woody plant); Seed bank persistence (1, transient; 2, short term persistent; 3, persistent); cluster number (see Table 3.4, App. 3.1).

The dissimilarity in species composition between the epi- and endozoochory samples was clearly reflected in the separation of both sample-groups along the first axis of the DCA-plot of all zoochory samples (Fig. 3.2). The second DCA-axis correlated strongest with date of sampling, indicating a seasonal variation in the species composition of the samples (Table 3.2). Also when the epi- and endozoochory samples were ordinated separately, the strong positive correlation of both first axes with date of sampling (Table 3.2) showed that time was a major factor explaining the variance within the epi- or endozoochory samples. The variation in axis scores among simultaneously collected samples illustrated the differences in species composition among seed loads of individual donkeys (Fig. 3.2).

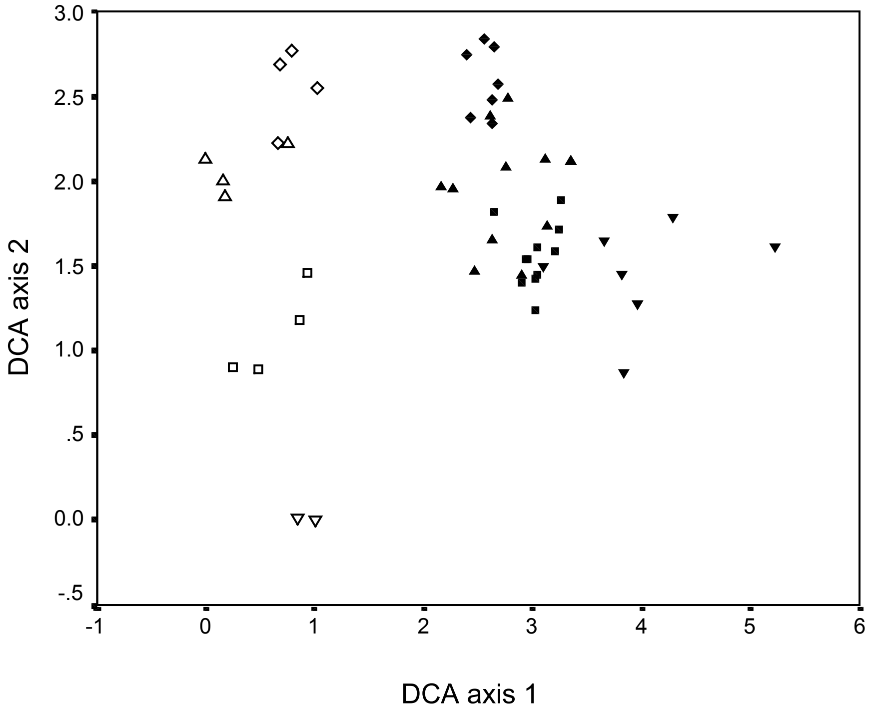


Fig. 3.2 *First two axes of Detrended Correspondence Analysis (DCA) of all zoochory samples (filled symbols, epizoochory samples; empty symbols, endozoochory samples; downward pointing triangles, early summer; squares, mid-summer; upward pointing triangles, late summer; diamonds, early autumn). The percentages of explained variance are shown in Table 3.2.*

Abundance in the study area

Species dominant in the study area (e.g. *Calamagrostis epigejos, Arrhenatherum elatius, Rubus caesius*) as well as rare species (e.g. *Leontodon saxatilis, Myosotis arvensis*) were dispersed zoochorously. The overall seedling density in the zoochory samples was positively correlated with species abundance in the study area (n=66, r_S=0.33, *P*=0.006). This correlation held true if only the species present in both epi- and endozoochory samples were considered (n=16, r_S=0.51, *P*=0.045), but not for the exclusively epizoochorous species (n=13, r_S=0.49, *P*=0.092) and the exclusively endozoochorous species (n=39, r_S=0.27, *P*=0.101).

Table 3.2. *Percentage of explained variance of the first four DCA axes and Spearman rank correlation coefficients (r_S) between sample scores and date of sampling, for a) epi- and endozoochory samples together (n=55), b) epizoochory samples (n=41) and c) endozoochory samples (n=14).*

DCA axis	zoochory		epizoochory		endozoochory	
	% of expl. var.	r_S	% of expl. var.	r_S	% of expl. var.	r_S
1	14.6	-0.55***	18.3	0.94***	20.4	0.77**
2	6.1	0.81***	7.4	0.05 ns	9.7	0.51 ns
3	3.5	0.45**	3.8	-0.08 ns	5.7	0.25 ns
4	2.8	0.23 ns	1.8	0.29 ns	1.9	0.46 ns

**** P<=0.001; ** P<=0.01; ns, not significant.*

Plant traits of zoochorous species and species in the study area

A number of plant traits differed between the species in the study area that were present in the zoochory samples and those that were not present in the samples (Table 3.3). The latter contained relatively more tall, woody perennials (trees and shrubs) with larger and heavier seeds (more berries and ant- or wind-dispersed seeds) and a transient seed bank. The group of species exclusively in the epizoochory samples contained relatively more biennial species, with adaptations for adhesive seed dispersal. The species exclusively in the endozoochory samples were generally smaller, mostly herbs, with smaller and lighter seeds, and a more persistent seed bank. Finally, the species in both epi- and endozoochory samples comprised relatively more grasses, often possessing 'unspecialized' seeds or seeds with adaptations to adhesive dispersal. The most significant differences existed between the species exclusively in the study area on the one hand, and the species exclusively in the endozoochory samples on the other hand, but this was probably partly due to the small size of the other groups.

Table 3.3 *Overview of the traits associated with the following groups: a) species in study area but not in zoochory samples; b) species exclusively in epizoochory samples; c) species exclusively in endozoochory samples; d) species both in epi- and endozoochory samples.*

		(a) exclusively study area	(b) exclusively epizoochory	(c) exclusively endozoochory	(d) epi+endo
number of species		269	13	37	16
seed weight (mg)	^{kw} 18.6**	30.7 b	3.9 ab	1.1 a	0.9 ab
seed length (mm)	^{kw} 16.2**	3.1 b	3.5 ab	1.7 a	2.0 ab
seed width (mm)	^{kw} 22.4***	1.8 b	1.6 ab	0.9 a	0.8 a
plant height (ordinal 1-8)	^{kw} 8.5*	3.4 b	3.1 ab	2.4 a	3.2 ab
life span (ordinal 1-4)	^{kw} 9.0*	3.3 b	2.3 a	3.0 ab	3.3 ab
dispersal strategy (categorical 1-6)	^{chi} 47.3*** (15)	-12/7/-1 2/4/-1	7/-2/-1 -1/-1/-3	2/-3/3 -1/-2/1	2/-2/-1 0/-1/2
reproduction type (categorical 1-4)	^{chi} 13.4 ns (12)	0/-4/1/3	0/6/-5/-1	0/-1/3/-1	0/0/1/-1
group (categorical 1-4)	^{chi} 35.2*** (9)	-8/0/-5/13	2/-1/1/-3	0/1/5/-7	5/-1/-1/-3
seed bank persistence (ordinal 1-3)	^{kw} 11.9**	2.2 a	2.2 ab	2.6 b	2.7 ab

Plant height (1, <100 mm; 2, 101-299 mm; 3, 300-599 mm; 4, 600-999 mm; 5, 1-3 m; 6, 3.1-6 m; 7, 6.1-15 m; 8, >15 m); Life span (1, annual; 2, biennial; 3, annual/biennial to perennial; 4, perennial); Dispersal strategy (1, adaptations for epizoochory; 2, ingested berries; 3, adaptations for anemochory; 4, adaptations for hydrochory; 5, adaptations for myrmecochory; 6, unspecialized); Reproduction type (1, only by seed; 2, mostly by seed; 3, by seed and vegetative; 4, mainly or exclusively vegetative); Group (1, grass; 2, sedge; 3, herb; 4, woody plant).

^{kw}*Kruskal Wallis test (values are group averages and groups that differ significantly are indicated by different letters);* ^{chi}*Pearson Chi*^{2} *assosiation test (values are the differences between the observed and the expected values for the different categories. A positive value indicates an overrepresentation of the category in the cluster). *** P<=0.001; ** P<=0.01; * P<=0.05; ns, not significant. Values between brackets are degrees of freedom.*

Dispersal-functional plant types associated with epi- and endozoochorous dispersal

Five dispersal-functional plant types were derived from cluster analysis of the 335 species in the study area on the basis of dispersal-relevant plant traits (Table 3.4, App. 3.1). Tall woody perennials (shrubs and trees) with large, heavy berries or wind-dispersed seeds and a transient seed bank were the main constituents of cluster 1. Cluster 2 predominantly consisted of biennial grasses and herbs with intermediately long seeds, adapted to epizoochory. Cluster 3 mainly grouped biennial herbs with light seeds, often with seed adaptations for dispersal by wind or ants, and cluster 4 contained predominantly perennial herbs with short seeds and various dispersal strategies. Biennial or perennial grasses, sedges and herbs with unspecialized seeds, often also reproducing vegetatively, were the main constituents of cluster 5. There was no significant difference in mean abundance in the study area between the member species of the different clusters. The species identified exclusively in the epizoochory samples nearly all belonged to cluster 2, while the species identified in both epi- and endozoochory samples had a broader distribution range. This range was even broader for the species occurring exclusively in the endozoochory samples.

Discussion

Zoochory in a coastal dune ecosystem

As demonstrated in this study, epi- and endozoochory are important dispersal mechanisms in coastal dune ecosystems grazed by large herbivores. At least one fifth of all species (66 of 335 species) in the study area were dispersed by the donkeys (Table 3.1). Probably even more species would have been revealed if more samples over a larger time span had been collected. In addition, the seedling emergence method may underestimate both species and seed numbers (see chapter 2). On the other hand, natural establishment rates of dispersed seeds may be considerably lower due to competition, predation and other environmental constraints (e.g. Edwards et al. 2000, Cosyns 2004).

The species composition of the epi- and endozoochory samples partially overlapped, but was clearly distinguishable in an ordination diagram (Table 3.1, Fig. 3.2). There was also a constant turnover in species composition of the zoochory samples in the course of the vegetation season (Table 3.2) (see also Malo & Suárez 1995a, chapter 2), and the abundance of the species in the samples was correlated with the abundance in the study area, at least for those species occurring in both epi-and endozoochory samples.

Trees and shrubs seemed, with the exception of *Rubus caesius*, not to be dispersed zoochorously (Tables 3.1 and 3.4, see also Fischer et al. 1995, Stender et al. 1997, Heinken et al. 2002), although fruits of e.g. *Ligustrum vulgare* and *Rosa pimpinellifolia* were seen bitten. Besides possibly unsuitable germination conditions, the relatively small amount of species with fleshy berries (e.g. *Rubus caesius, Solanum nigrum*) germinating from the donkey dung, may also suggest that these species are better adapted for dispersal by frugivorous birds than by large herbivores. Further investigation may elucidate this. A number of other plant traits differed between the species in the study area which were present in the zoochory samples, and those which were not (Table 3.3), and between the different clusters associated with the different types of zoochory (Table 3.4). Morphological seed properties such as seed size and seed weight seemed important indicators for zoochorous dispersal, as well as special dispersal adaptations. The latter seemed more indicative in the case of epizoochory than in the case of endozoochory. Still, a much wider range of dispersal adaptations was represented among the epi- and endozoochorously dispersed species than previously assumed (see Grime et al. 1988, Table 3.1). For endozoochory, small and light seeds, a small plant size and a persistent seed bank (see also Pakeman et al. 2002) seemed important factors, while adhesive seed adaptations and a short life span seemed associated with epizoochory. The species identified in both epi- and endozoochory samples often possessed intermediate traits.

Pakeman et al. (2002) suggested that many species adapted to endozoochory by large herbivores might be hidden in the dispersal strategy category ‘unspecialized’ (Grime et al. 1988). These species lack obvious seed adaptations and use their edible vegetative parts as ecological ‘fruits’ to attract herbivores as seed dispersers. Janzen (1984) formulated this ‘foliage is the fruit’ hypothesis in the context of endozoochory. However, the presence of many grasses with unspecialized seeds in our epizoochorory samples (Table 3.1, App. 3.1, see also chapter 2) may suggest that the ‘foliage is the fruit’ hypothesis might not only apply to endozoochory, but also to epizoochory. Apparently, not only specialized seed adaptations increase the chance of dispersal in the fur of herbivores, but also increasing the chance that animals come into contact with the seeds, by providing a food source. The fact that the inflorescence of many grasses is physically separated from the edible leaves by a tall stem, may further enhance the probability of adherence of the seeds to the fur of grazing herbivores. Edibility might thus enhance both forms of zoochory.

Table 3.4 *Overview of the traits used to define dispersal-functional plant type clusters (named by their predominant profile). The abundance of the species in the study area is given, as well as the number of species identified in the exclusively epizoochory samples, the exclusively endozoochory samples, or in both. The last three rows show the percentage of the total number of cluster species in the former groups.*

cluster number		1	2	3	4	5
dispersal-functional type		Tall woody perennials with large, heavy seeds (berries or wind-dispersed) and a transient seedbank	Biennial grasses and herbs with intermediately long seeds adapted to epizoochory	Biennial herbs with light seeds adapted to dispersal by wind and ants	Perennial herbs with short seeds and various dispersal strategies	Biennial or perennial grasses, sedges and herbs with unspecialized seeds, often also reproducing vegetatively
n° of species in cluster	total = 335	74	63	51	77	70
seed weight (mg)	^{kw} 58.6***	98.07 b	2.93 a	1.39 a	13.64 a	2.90 a
seed length (mm)	^{kw} 62.0***	4.93 c	3.13 b	2.34 ab	1.90 a	2.33 ab
seed width (mm)	^{kw} 66.3***	3.29 b	1.29 a	1.13 a	1.14 a	1.25 a
plant height (ordinal 1-8)	^{kw} 144.7***	5.72 b	2.51 a	2.51 a	2.53 a	2.53 a
life span (ordinal 1-4)	^{kw} 114.8***	4.00 c	2.61 ab	2.19 a	3.92 c	2.97 b
seed bank persistence	^{kw} 35.4***	1.38 a	2.19 b	2.55 b	2.47 b	2.40 b
dispersal strategy (categorical 1-6)	^{c} 540.8*** (20)	-11/29/6/-1/-6/-17	36/-7/-14/-3/-4/-8	-10/-7/24/1/10/-18	-4/-5/4/4/2/-1	-11/-10/-19/-1/-3/44
reproduction type	^{c}236.2*** (16)	1/-2/1/0	19/-16/-2/0	27/-23/-4/0	-44/47/-5/2	-2/-7/10/0
group (categorical 1-4)	^{c} 374.8*** (12)	-6/-1/-46/54	14/0/-4/-11	-6/-3/22/-13	-9/2/23/-16	7/3/5/-14
abundance in the study area	^{kw} 7.0 ns	1.86 a	2.14 a	2.06 a	1.84 a	2.06 a
sp. excl. in epi		0	10	3	0	0
sp. excl. in endo		2	6	9	12	8
sp. excl. in epi+endo		0	5	3	2	6
% cluster sp. excl. in epi		0	16	6	0	0
% cluster sp. excl. in endo		3	10	18	16	11
% of sp. in epi+endo		0	8	6	3	9

Plant height (1, <100 mm; 2, 101-299 mm; 3, 300-599 mm; 4, 600-999 mm; 5, 1-3 m; 6, 3.1-6 m; 7, 6.1-15 m; 8, >15 m); Life span (1, annual; 2, biennial; 3, annual/biennial to perennial; 4, perennial); Seed bank persistence (1, transient; 2, short term persistent; 3, persistent); Dispersal strategy (1, epizoochory; 2, ingested berries; 3, anemochory; 4, hydrochory; 5, myrmecochory; 6, unspecialized); Reproduction type (1, only by seed; 2, mostly by seed; 3, by seed and vegetative; 4, mainly or exclusively vegetative); Group (1, grass; 2, sedge; 3, herb; 4, woody plant); Abundance in the study area (1, present; 2, occasional; 3, frequent; 4, codominant; 5, dominant).

^{kw} *Kruskal Wallis test (values are cluster averages and clusters that differ significantly are indicated by different letters);* ^{c} *Pearson* Chi^2 *association test (values are the differences between the observed and the expected values for the different categories. A positive value indicates an overrepresentation of the category in the cluster). ***P<=0.001; ns, not significant. Values between brackets are degrees of freedom.*

Complementarity of epi- and endozoochory

The differences in species composition between the epi- and endozoochory samples, as well as the different association of dispersal-functional plant types with either of the mechanisms, demonstrated the complementarity of epi- and endozoochory. This complementarity seemed to be more pronounced than the additive effect of epi- and endozoochory, since only 16 species were dispersed by both mechanisms and 50 species by only one of the mechanisms. In addition, the different association of epi- and endozoochory with the dispersal-functional plant types revealed an increasing degree of specificity from endozoochory towards epizoochory, since the endozoochorously dispersed species covered many more dispersal-functional plant types than those dispersed by epizoochory. Although endozoochory is supposed to have a greater impact than epizoochory on vegetation dynamics in terms of quantity of seed dispersal (Janzen 1984), both mechanisms are ecologically important and complementary. The combination of epi- and endozoochory results not only in a larger number of dispersed seeds, but, more importantly, in a larger spectrum of dispersed species. This is a consequence of the different constraints of both mechanisms. Whereas e.g. grooming behaviour of animals can limit epizoochory (Sorensen 1987, Kiviniemi 1996), endozoochorous dispersal is constrained by seed survival in the digestive tract (Neto & Jones 1987, Gardener 1993, Cosyns et al. in press b) and by feeding preferences of herbivores. Yet, the general relative ecological significance of both dispersal modes as compared to each other but also compared to other possible long-distance seed dispersal modes needs further investigation, taking plant recruitment into account.

From an evolutionary point of view, the complementarity of epi- and endozoochory can be seen as a result of different selective forces operating on plants. The mechanistics of epi- and endozoochory are very different, hence different seed adaptations will be favoured by either process. Seeds with resistent seed coats are more likely to pass undamaged through the molar mill and the digestive tract of herbivores (Pakeman et al. 2002, Cosyns et al. in press b), while adhesive seed adaptations should be more successful in terms of epizoochorous dispersal (see also chapter 4). Selective pressure in the context of endozoochory is also directly related to the feeding preferences of the dispersal vector. Seeds that are not eaten cannot disperse via endozoochory, so species which can attract herbivores by offering a food source (fleshy berries or palatable vegetative organs, see Janzen 1984) increase their chance of dispersal. Epizoochory, at the contrary, is generally considered an ‘accidental’ form of dispersal, because animals carrying seeds in their fur do not gain any nutritional reward (Sorensen 1986). Sorensen (1986) and Kiviniemi (1996) demonstrated that large and irritating seeds are removed faster by animals than small and inconspicious seeds. Thus, adhesive adaptations may turn out contraproductive when too irritating to animals, while small seeds may be very successful in epizoochorous dispersal (see also chapter 2). Selective pressures related to different dispersal mechanisms may also operate simultaneously on plant species. Many grass species, for example, may be dispersed by endozoochory, epizoochory, and anemochory. Edible vegetative parts, in combination with resistent seed coats, an adhesive awn and a long stem may enhance the probability of all three mechanisms. This illustrates the complexity of evolutionary pressures possibly operating on plants. However, a solid interpretation of plant traits in terms of adaptation to dispersal is difficult, because the ultimate impact of seed dispersal on plant recruitment is very difficult to assess, and obviously related to the spatial configuration of suitable habitat patches. Dispersal may lead to escape from density-dependent mortality, to colonization of distant patches, or to directed dispersal towards favourable habitats (Howe & Smallwood 1982). Zoochory is assumed to contribute especially to the latter (Wenny 2003).

Consequences for nature mangement

As large herbivores have always been part of natural ecosystems, their complementary role as epi- and endozoochorous dispersal vectors may be important for maintaining species richness. Grazing by livestock has recently become an important nature management tool for conservation and restoration of many habitats in northern temperate regions (see also chapter 2). Until now, much attention was given to the contribution of livestock to plant diversity patterns, both at the local and landscape scale, through their activities of selective grazing, trampling and defecating, which influence processes that enhance local extinction rates. Yet herbivores may also influence plant diversity through processes that affect colonisation rates (Olff & Ritchie 1998). The results of this paper clearly show the importance of large herbivores as long-distance seed dispersal vectors for much more plant species than could be assumed from their morphological seed characteristics (see also chapters 2, 4, 5). Retention times of seeds in the fur (see chapter 5) and in the digestive tract of large herbivores (Cosyns et al. in press b) allow dispersal over distances of metres to kilometers, hence covering the entire 80 ha study area (see also chapter 6). The growing evidence for the role of large herbivores in the seed dispersal process urges for a well-considered nature management policy which not only focuses on amelioration of habitat conditions, but also considers the spatial arrangement of suitable but still unoccupied patches for critical plant species. If plants can bridge gaps in space and time, this may favour a sustainable conservation of critical plant populations. It is shown here that the use of large herbivores like donkeys as managers, might help to reach this goal, through the epi- and endozoochorous dispersal of plant seeds.

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App. 3.1. *All plant species in the study area, clustered in dispersal-relevant functional plant types. Species are represented by the first four letters of genus resp. species name (first four letters in capitals, species exclusively in epizoochory samples; last four letters in capitals, species exclusively in endozoochory samples; all letters in capitals, species both in epi- and endozoochory samples).*

Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
acerpseu	rosarugo	agrieupa	trifcamp	ancharve	conyCANA	achiMILL	mentaqua	aegopoda	stelpall
popucand	sympalbu	CYNOoffi	trifDUBI	fumaoffi	sisyoffi	vincmajo	apiurepe	allivine	gerapusi
tiliplat	cotohori	GEUMurba	BIDEtrip	lamipurp	senevisc	epillanc	ranuflam	fallaube	veropers
fraxexce	rubuCAES	ranuacri	GALIAPAR	mercannu	senevulg	sedurupe	ranuREPE	cardprat	vicilath
carpbetu	rubufruc	helinumm	ARCTminu	verohede	SONCaspe	ceratome	carepani	convarve	anagarve
ulmmino	cotosimo	ballnigr	arctpube	clayperf	SONCOLER	leonSAXA	hydrvulg	lemnmino	stelMEDI
betuspp	ribeuvac	RUMEobtu	MYOSarve	lamiampl	lactserr	orobcary	lysivulg	potacris	matrmatr
pinunigr	hedeheli	galiMOLL	torijapo	lamihybr	senesylv	pyrorotu	dantdecu	ranutric	vicihirs
popualba	rosapimp	planLANC	erodlebe	anchoffi	carlvulg	hierpilo	luzucamp	orniumbe	fallconv
popubals	mahoaqui	PLANMAJO	myosramo	chelMAJU	cirspalu	seduacre	juncARTI	ranufica	polypers
popucans	prunspin	ranubulb	myosstric	theshumi	pastsati	veroanag	juncsubn	AGROCAPI	fumamura
salialba	cratmono	thympule	planCORO	polyserp	SENEJACO	epilangu	scirseta	koelALBE	chenALBU
pinusylv	prunsero	juncinfl		polyvulg	cirsvulg	hierumbe	bellpere	agroSTOL	chenrubr
clemvita	pyrucomm	PHLEPRAT		violcani	crepbien	pulidyse	poteanse	HOLCLANA	vicisati
salimult	prunaviu	anthodo		violcurt	OENObien	EPILCILI	nastoffi	milieffu	anthsylv
salicapr	pruncera	avenpube		bryodioi	oenoGLAZ	seneeruc	rumeCRIS	POAPRAT	chaetemu
ammoaren	euoneuro	ARRHelat		solaNIGR	verbthap	cirsarve	thalminu	POATRIV	centthui
phraaust	rosacaes	triseflav		erigacer	dipltenu	sapooffi	poteerec	elumobtu	rumeprat
calaEPIG	franalnu	holcmoll		tragprat	taraspp	epilHIRS	poterept	elymath	lathprat
humulupu	rosacani	DACTglom		heraspho	silenuta	eupaCANN	cardhirs	glycnota	viccCRAC
salirepe	vibulant	festarun		LYTHSALI	hypoRADI	epilMONT	ceraarve	glycflui	lithoffi
syrivulg	malusylv	festgiga		rumecong	siledioi	hypeperf	ceraFONT	poapalu	aquivulg
poputrem	sorbauc	festfili		myoscaesp	silevulg	lychflos	rumeacet	festjunci	centjace
salifrag	liguvulg	lolipere		ranuscel	sileLATI	epiphell	veroCHAM	FESTRUBR	centnigr
saliatro	rosatome	molicaer		rumemari		hypetetr	verooffi	careAREN	ARTEVULG
salicine	rosaobtu	POAANNU		arabthal		inucony	potester	careflacc	aspecyna
alnuglut	rosarubi	airaPRAE		gnaplute		arumital	saginodo	elymrepe	sagiPROC
alnuinca	ribealpi	bromthom		capsBURS		aspaoffi	galaniva	leymaren	lotucorn
cladmari	ribenigr	PHLEaren		centeryt		sambebul	lamigale	caredist	arabhirs
eleopalu	ribeodor	juncBUFO		saxitrid		soladulc	lamialbu	carehirt	jasimont
coluarbo	riberubr	aperinte		centpulc		groendens	lamimacu	caretrin	moehtrin
labuanag	ribesang	BROMhord		CERAsemi		calysepi	glechede	careviri	
ononrepe	sambnigr	hordmuri		sileconi		hemespp	violhirt	allipeti	
rhushirt	prundome	bromtect		crepCAPI		galiulig	myosscor	chenmura	
robipseu	querrobu	bromster		centlitt		rheum	prunvulg	geraMOLL	
cornsang		ANTHCAUC		diplmura		galiVERU	trifREPE	gerarober	
lycibarb		urtiuren		eropvern		thalflav	zannpalu	ceradiff	
rubuidae		erodcicu		arenSERP		apiunodi	URTIDIOI	veroarve	
hipprham		myosdisc		corycane		galipalu		cochdani	

Cluster legend:
1, Predominantly tall woody perennials with large, heavy seeds (berries or wind-dispersed);
2, Predominantly biennial grasses and herbs with intermediately long seeds, adapted to epizoochory;
3, Predominantly biennial herbs with light seeds, adaptated for dispersal by wind and ants;
4, Predominantly perennial herbs with short seeds and various dispersal strategies;
5, Predominantly biennial or perennial grasses, sedges and herbs with unspecialized seeds, reproducing by seed or vegetatively.

Chapter 4

An experimental assessment of seed adhesivity on animal furs

Abstract

Epizoochory is widely recognized as an effective long-distance seed dispersal mechanism. Nevertheless, few studies focus on the investigation of its influencing factors. One of the key aspects of epizoochory is the adhesive interaction between seeds and furs. We describe a new method to quantify experimentally and standardize the adhesivity of seeds to animal fur, as a measure of epizoochorous dispersal potential. The method excludes the impact of animal behaviour and environmental factors, and allows the ranking of species according to their adhesivity score. We measured adhesivity scores for 66 species on the furs of seven mammals. Deep furs with long, rough, undulated hairs implanted at a large angle were most suited for seed adhesion, while seeds adhered less well to shallow furs with short, smooth, straight hairs implanted at small angles. Seeds with specialized adhesive appendages had higher adhesivity scores than seeds with unspecialized appendages and seeds without appendages. However, the functionality of certain seed traits varied with fur type (e.g. specialized adhesive appendages did not enhance the adhesivity on fur of wild boar). Although seed morphology was a good predictor for seed adhesivity on fur, less well-adhering seed types often still had relatively high adhesivity scores. Therefore, it is likely that nearly all species are, to some extent, able to disperse epizoochorously.

Key words

Adhesive seed dispersal, adhesivity score, epizoochory, mammalian fur, methodology, seed traits

Introduction

Dispersal of plant seeds in the fur of animals (epizoochory) is presumed to be one of the key factors in historical and actual long-distance seed transport, but is far from being fully understood (Bonn & Poschlod 1998, Higgins & Richardson 1999, Higgins et al. 2003a, b). In the past, seed dispersal mechanisms were mainly derived from morphological characteristics of seeds (cf. Grime et al. 1988, Hughes et al. 1994). However, the idea that seeds are merely dispersed by one process, i.e. the process they are morphologically adapted for, is no longer tenable. It is now recognized that seeds may disperse by several dispersal mechanisms (Higgins et al. 2003b).

To identify these mechanisms, observational studies in the field are extremely valuable, because they provide evidence for the occurrence of a certain dispersal mode in combination with certain plant species. In the case of epizoochory, such field studies comprise inspection of the epizoochorous seed loads of animals (see Agnew & Flux 1970, Shmida & Ellner 1983, Stender et al. 1997, Fischer et al. 1996, Mrotzek et al. 1999, Heinken 2000, Heinken & Raudnitschka 2002, Graae 2002, Schmidt et al. 2005, chapters 2 and 3). However, to unravel the process behind these observations, more directed experiments are indispensable. For instance, field experiments in which marked seeds are attached to living animals allow control of the attachment procedure, but have the disadvantage that the influence of environmental (e.g. vegetation, weather) and behavioural factors (e.g. animal movement, grooming, wallowing) cannot be entirely separated from the dispersal and detachment process (see Shmida & Ellner 1983, Sorensen 1986, Stender et al. 1997, Fischer et al. 1996, Kiviniemi 1996). Even if these experiments are conducted in laboratory conditions, the behavioural aspects of the animals still interfere with the behaviour of the seeds (e.g. noticeable or irritating seeds can induce grooming behaviour, Kiviniemi & Telenius 1998). The use in the field of man-made constructions, such as dummies, can allow the researcher to control the behavioural factor, but not the vegetational or other environmental influences (see Bullock & Primack 1977, Fischer et al. 1996, Heinken et al. 2001, Castillo-Flores & Calvo-Irabien 2003). To fully understand the process of seed dispersal, it is therefore necessary to study the affecting factors separately, i.e. to raise the level of experimental control. Until now, few studies include such highly controlled experiments. They provide, however, missing pieces of the puzzle, since only an integration of field observations and experimental data, which isolate the different influencing factors, can result in a full understanding of the dispersal process.

One of the most obvious aspects of epizoochorous seed dispersal is the adhesive interaction between seeds and furs. Experimental quantification of this interaction requires a standardized seed attachment procedure, in which environmental factors and animal behaviour are controlled. To our knowledge, no such experiments are described in the literature. Only Lacey (1981) mentioned a limited experiment with *Daucus carota* seeds and prepared mammalian furs, and Gorb & Gorb (2002) measured the contact separation force of burrs to assess the mechanical interlocking ability of four plant species. As such, there is a considerable gap in our knowledge with respect to this key aspect of epizoochorous seed dispersal.

In this study, we investigate the adhesive interaction of different seed types with the furs of different mammals. A useful quantitative measure of seed adhesivity to fur, as a key aspect of epizoochorous dispersal potential, should allow species to be ranked on an ordinal scale with regard to their adhesive properties. Besides the possibility of comparing the epizoochorous dispersal potential among species, the advantage of such a ranking is the feasibility of comparing the relative dispersal potential of species with regard to several dispersal modes, at least if comparable methods for these other dispersal modes exist (see also Tackenberg et al. 2003). In an attempt to develop such a method in the context of epizoochory, we designed a standardised method to quantify seed adhesivity to samples of animal furs in controlled conditions. The following research questions were adressed:

- Does seed adhesivity differ among different fur types?
- Which fur traits enhance the adhesivity of seeds?
- Is seed morphology predictive for the adhesive behaviour of seeds?
- Can all types of seed appendages enhance adhesivity to fur?
- Is there an interaction-effect between fur type and seed morphology with respect to adhesivity?

Material and methods

Species selection and seed characterization

Diaspores of 66 plant species were collected in the field (usually seeds or fruits, but further referred to as seeds, see App. 4.1). The selected species covered a broad range of morphological seed features, and had either been reported in the literature as dispersing epizoochorously (see App. 4.1), or were chosen because of their commonness and availability in the western-European landscape.

Because intra-specific variation in seed size occurs frequently and since accurate measurements are relatively scarce in the literature, eight morphological traits relevant for adhesive dispersal were carefully recorded (see App. 4.1): (1) type of adhesive appendage: hooks (hooked appendages), bristles (straight appendages), awns (organ hypothesized to anchor seeds into the soil, present on diaspores of many grass species), hairs (pappus hairs or hairs covering the seed surface), stems or remnants of the perianth, no appendages; (2) seed-surface texture rank: smooth, slightly rough, rough, hairy, sticky; (3) rank of appendage density (no appendages, 1 appendage, low density, high density). The continuous variables (4) appendage length, (5) seed length (excluding appendages), (6) seed width, and (7) seed thickness were measured as the average of 15 randomly chosen seeds, while (8) seed weight was calculated by dividing the weight of 50 seeds by 50. To assign all species to one of the appendage type categories, a limited number of simplifications was necessary. Seeds with more than one type of appendage were assigned the type of the most noticeable one, e.g. for *Anthoxanthum odoratum,* the awn was chosen as most prominent adhesive appendage, while the hairs present on the dispersal unit were incorporated in the surface texture rank. For *Juncus effusus* diaspores, the stem was considered the adhesive appendage, while the surface texture category was described as ‘rough’ because of the presence of spiny *tepala*.

Fur preparation and characterization

Prepared fur samples of seven mammalian species were collected, among which are some important wild animals in our region -roe deer (*Capriolus capriolus*), wild boar (*Sus scrofa*) and rabbit (*Oryctolagus cuniculus)*- as well as some common domesticated animals in agriculture and nature management: horse (*Equus caballus*), sheep (*Ovis aries)* (a meat race) and two races of cattle (*Bos taurus*): Holstein cattle (short-haired) and Galloway cattle (long-haired). Most of these animals had already been reported in the literature to be important seed dispersers (see App. 4.1).

Because intra-specific variations in fur traits, e.g. the contrast between summer and winter fur, are sometimes even more pronounced than some inter-specific differences, we recorded seven fur traits to obtain an objective fur description. We measured (1) the length of the individual hairs and (2) the depth of the fur (without straightening the hairs). Additionally, a rank order was assigned to (3) the thickness of the individual hairs, (4) the density of the hair implantation, (5) the roughness of the fur, (6) the hair undulation, and (7) the angle between hair and skin (App. 4.2).

Comparison of the furs with those of living animals indicated that the prepared fur samples were somewhat softer or less greasy than the fur of living animals. Therefore, in order to imitate the original roughness, the fur samples were rubbed with a small amount of moist loamy sand, which was brushed out again after drying.

Adhesivity tests

To estimate the seed adhesivity to the different fur samples, a simple test was designed, in which both seed application and fur manipulation were standardized. To facilitate manipulation of the furs, they were clasped between two wooden boards, one of which had a gap of 25 x 25 cm, thus leaving a 25 x 25 cm zone of fur uncovered (see App. 4.3). To put the seeds on this test zone, the construction was placed on an horizontal surface, the test zone facing upwards. For each test, 50 seeds of a certain species were dropped perpendicularly on the fur, from a height of 15 cm above the test zone. Care was taken to spread the seeds more or less homogeneously over the test zone so that they would not hamper each others' attachment. Subsequently, the wooden construction was carefully lifted upwards, rotated to an upside-down position above a collection box and turned back into the original position, slowly and without irregular movements. The rotation of the construction was always in the direction of the hair implantation, to avoid retrapping of falling seeds in the fur. The seeds that fell off were counted and those that were still attached removed. This test was repeated five times for each seed-fur combination. The proportion of attached seeds was then averaged for the five tests, resulting in an 'adhesivity score' between 0 and 1 for each seed-fur combination.

Data analysis

For all seed species, the adhesivity score on each of the seven furs was calculated, as well as the 'global adhesivity score' (the mean adhesivity score on all furs). The data were then analysed to reveal the differences between the seven animal furs, the importance of the fur traits and the role of the seed traits. Only if the assumptions for the use of parametric statistics were not met, non-parametric statistics were used and, unless mentioned otherwise, all statistical analyses were performed using SPSS 10.0 (SPSS 1999). For multiple comparisons between groups, a least significant difference (LSD) correction with alpha = 0.05 was performed (Siegel & Castellan 1988).

Firstly, the seven fur samples were compared using Spearman rank correlation coefficients (Siegel & Castellan 1988) between the adhesivity scores on the different furs. Additionally, the mean adhesivity scores of the furs were tested for significant differences between the furs with a Kruskal Wallis test, followed by multiple comparisons (Siegel & Castellan 1988). To visualize the position of the furs in a two-dimensional space, we applied Principal Component Analysis (PCA) on the adhesivity scores, using the program CANOCO 4.5 (ter Braak & Smilauer 2003). The fur traits were then plotted as 'supplementary environmental variables' on the ordination diagram.

Secondly, the role of the seed traits was examined. Spearman rank correlation coefficients were calculated between the values of the seed traits and the adhesivity scores (individual and global). This was done for the complete set of species and for the set of species without adhesive appendages, to separate the influence of the presence of adhesive appendages from the influence of the other seed traits. For the nominal variable 'appendage type', a one-way ANOVA with *post hoc* multiple comparisons (LSD) was performed to test whether the global adhesivity scores differed between seeds with different appendage type categories.

Then, the seed species were clustered into more homogeneous groups on the basis of the seed traits, using the Gower's similarity coefficient and the 'increase in sum of squares' method, using the program Clustan Graphics 5.08 (Clustan Ltd. 2001). The resulting seed clusters were then characterized in function of the seed traits, using Kruskall Wallis tests in combination with multiple comparisons for the continuous and ordinal seed traits, and a Pearson Chi^2 association test for the nominal variable (Siegel & Castellan 1988). The most distinctive trait was incorporated in the cluster names.

Subsequently, to check whether the seed clustering could account for the difference in seed adhesivity, a multivariate analysis of variance (MANOVA) was used, with seed clusters as factor and adhesivity on the different furs as dependent variables. In addition, a between-subjects univariate test (one-way ANOVA) and *post hoc* multiple comparisons (LSD) were performed to reveal significant differences in adhesivity scores between the clusters on individual furs. To homogenize the variances, the variables 'adhesivity on horse' and 'adhesivity on rabbit' were square root-transformed, while for the other furs the assumption of homogeneity of variances between the seed clusters was fulfilled.

Finally, Discriminant Analysis (DA) (Dillon & Goldstein 1984) was used to examine the degree to which the division of the seed species by the cluster analysis, which resulted solely from the seed traits, matched the pattern of adhesivity to the furs. The adhesivity scores on the different furs were used as predictor variables and the cluster division as grouping variable in the DA. The percentage of seed species correctly assigned to a seed traits-based cluster indicates the strenght of the association of the seed characteristics with the adhesivity scores. The DA-ordination plot, based on adhesivity scores, was compared to a NMDS (nonmetric multidimensional scaling, Doyle 1973) ordination plot, based solely on the seed traits. For this NMDS ordination, the Gowers' proximity matrix computed in Clustan Graphics 5.0 was used.

Results

The adhesivity scores of the 66 seed species on the different fur samples ranged from 0.000 to 0.876 (App. 4.2). The global adhesivity ranged from 0.003 to 0.629, with a mean of 0.27.

Comparison of furs and role of fur traits

The adhesivity scores were highly positively correlated among the furs (r_S ranging from 0.62 to 0.92, P<0.001). However, the correlation was somewhat lower if wild boar was one of the compared furs (data not shown). The adhesivity scores differed significantly between the furs (Kruskal Wallis test: χ^2=156.88; df=6; *P*<0.001) (Table 4.1).

Table 4.1 *Mean adhesivity scores (n=66) on the seven furs (Kruskal Wallis test: χ^2=156.88; df=6; P<0.001). Different letters indicate significant differences between groups. The global adhesivity scores, calculated as the average of the mean adhesivity scores of all furs together, ranged from 0.003 to 0.629, with a mean of 0.27.*

Fur	mean	range
Horse	0.12 a	0.000-0.464
Rabbit	0.14 a	0.000-0.628
Holstein	0.19 ab	0.000-0.496
Roe deer	0.26 bc	0.000-0.720
Wild boar	0.35 c	0.000-0.596
Sheep	0.37 c	0.000-0.788
Galloway	0.50 d	0.004-0.876

The adhesivity scores on the furs of horse, rabbit and Holstein cattle were significantly lower than those on wild boar, sheep and Galloway cattle. Roe deer had an intermediate position, with adhesivity scores not significantly higher than Holstein cattle nor lower than those on wild boar and sheep. The adhesivity scores recorded on Galloway cattle were still significantly higher than those on sheep and wild boar. This ranking was also reflected in the position of the furs along the first axis of the PCA-ordination plot based on the adhesivity scores (% of explained variance, axis 1: 79.3%; axis 2: 7.4%) (Fig. 4.1). The fur traits (App. 4.2) positively correlated with PCA-axis 1 were hair length, fur depth, fur roughness, hair undulation and hair/skin angle. Hair thickness and fur density were positively and negatively correlated, respectively, with the second axis (explaining much less of the total variance than axis 1).

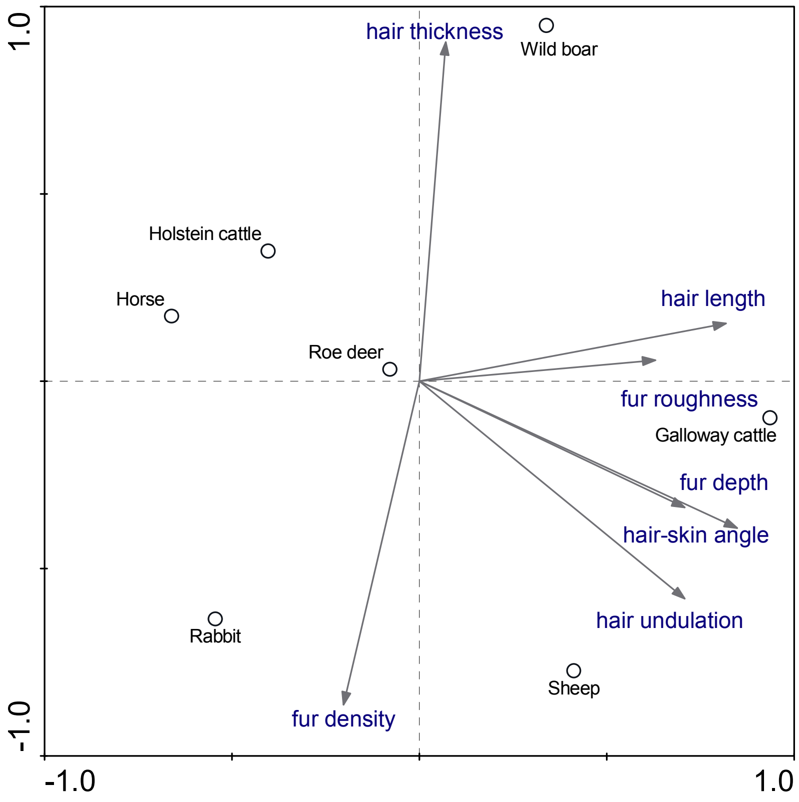


Fig. 4.1 *Principal component analysis (PCA) of the seven fur samples, based on the adhesivity scores of 66 seed species (79.3% of the total variance is explained by axis 1 and 7.4% by axis 2). The fur traits were plotted as 'supplementary environmental data'.*

Role of seed traits and functional seed groups

The adhesivity scores (App. 4.1, Table 4.2) were consistently significantly negatively correlated with seed length, seed width, seed thickness and seed weight, especially if only the seed species without adhesive appendages were considered. The surface texture, on the other hand, was only weakly positively correlated with adhesivity on some furs, if all seed species were included in the analysis. The appendage length and density showed a weak, but significant, positive correlation with adhesivity, except for wild boar. The appendage types associated with the highest global adhesivity scores (Table 4.3) were awns, followed by hooks and then bristles. Hairs seemed to be intermediately efficient, and stems or perianth remnants even less efficient than no appendages.

Table 4.2 *Spearman rank correlations between adhesivity scores on different furs and seed traits.*

	seed length	seed width	seed thickness	seed weight	surface texture	appendage length	appendage density
Horse[1]	-0.30*	-0.57***	-0.54***	-0.69***	0.31*	0.33**	0.29*
Rabbit[1]	-0.32*	-0.54***	-0.42***	-0.61***	0.38**	0.33**	0.34**
Holstein cattle[1]	-0.43***	-0.58***	-0.50***	-0.68***	0.26*	0.26*	0.28*
Roe deer[1]	-0.48***	-0.70***	-0.57***	-0.79***	0.20ns	0.26*	0.26*
Wild boar[1]	-0.57***	-0.67***	-0.49***	-0.74***	0.14ns	-0.08ns	-0.09ns
Sheep[1]	-0.32**	-0.52***	-0.38**	-0.59***	0.41**	0.35**	0.35**
Galloway cattle[1]	-0.30*	-0.55***	-0.42***	-0.63***	0.31*	0.29*	0.29*
Global adhesivity[1]	-0.39**	-0.63***	-0.50***	-0.72***	0.33**	0.31*	0.31*
Horse[2]	-0.59***	-0.75***	-0.80***	-0.84***	0.23ns		
Rabbit[2]	-0.69***	-0.71***	-0.59***	-0.80***	0.22ns		
Holstein cattle[2]	-0.73***	-0.70***	-0.67***	-0.83***	0.21ns		
Roe deer[2]	-0.81***	-0.81***	-0.70***	-0.93***	0.08ns		
Wild boar[2]	-0.75***	-0.80***	-0.64***	-0.84***	0.18ns		
Sheep[2]	-0.79***	-0.74***	-0.60***	-0.85***	0.23ns		
Galloway cattle[2]	-0.61***	-0.71***	-0.58***	-0.82***	0.11ns		
Global adhesivity[2]	-0.81***	-0.85***	-0.68***	-0.96***	0.17ns		

[1] *all seed species included in the analysis (n=66).*

[2] *only seed species without adhesive appendages included (n=37).*

**** P<=0.001; ** P<=0.01; * P<= 0.05; ns, not significant.*

Table 4.3 *Global seed adhesivity scores in different classes of seed appendage type. A one-way ANOVA (F=8.66, df=5, P<0.001) was calculated to test the differences between the classes. Values are group averages and groups that differ significantly are indicated with different letters.*

appendage type	n	global adhesivity	standard deviation
stem, perianth remnants	9	0.155 a	0.101
no appendages	37	0.233 a	0.120
hairs	10	0.329 b	0.182
bristles	2	0.414 bc	0.086
hooks	4	0.464 bc	0.015
awns	4	0.530 c	0.080

Three seed clusters resulted from the cluster analysis based on the seed traits (cluster members in App. 4.1). All traits except seed thickness and seed weight differed significantly between at least two of the clusters (Table 4.4). The first seed cluster was characterized by rather long, broad seeds with one appendage (stem or perianth remnants). The second cluster contained, on average, rather long, narrow seeds with a rough surface texture and a low to high density of appendages (hairs, awns, bristles or hooks). Finally, the third cluster comprised the rather short, narrow, smooth textured seeds without appendages. The three clusters were named, respectively, 'SUA' (seeds with unspecialized appendages), 'SSA' (seeds with specialized appendages) and 'SWA' (seeds without appendages).

The effect of seed cluster on adhesivity for all furs together was highly significant (MANOVA, Pillai's Trace, F = 3.31, df = 14, *P*=0.0002). For most furs, the adhesivity of seed cluster SSA was significantly higher than that of cluster SUA and SWA (one-way ANOVA and multiple comparisons, see Table 4.5). Two furs, however, behaved differently. The adhesivity on Galloway differed significantly between all three clusters, cluster SWA taking an intermediate position between SUA and SSA. For wild boar, the adhesivity of cluster SWA was as high as that of cluster SSA.

Table 4.4 *Overview of the seed traits associated with the three seed clusters (SUA, seeds with unspecialized appendages; SSA, seeds with specialized appendages; SWA, seeds without appendages; n= number of seed species in the clusters).*

seed trait	test statistic	SUA (n=9)	SSA (n=20)	SWA (n=37)
seed length (mm)	^{kw} 13.99***	9.23 a	4.37 a	3.06 b
seed width (mm)	^{kw} 6.40*	3.2 a	1.12 b	1.42 ab
seed thickness (mm)	^{kw} 3.75 ns	2.14 a	0.83 a	0.90 a
seed weight (mg)	^{kw} 4.54 ns	9.84 a	1.76 a	2.01 a
surface texture (rank)	^{kw} 7.72*	2.11 ab	3.15 a	1.86 b
appendage length (mm)	^{kw} 58.57***	2.62 a	5.02 a	0.00 b
appendage density (rank)	^{kw} 61.57***	1.00 a	2.20 a	0.00 b
appendage type	^{chi}132***	-5.0/7.8/-0.5/ -1.4/-0.3/-0.5	-11.2/-2.7/2.8/ 7.0/1.4/2.8	16.3/-5.0/-2.2/ -5.6/-1.1/-2.2

^{kw} *Kruskal-Wallis test. Values are group averages and groups that differ significantly are indicated with different letters.* ^{chi} *Pearson Chi*^{2} *association test. Values are the differences between the observed and expected values for the different appendage types (First line: no appendages / stem or remnants of perianth / awn; Second line: hairs / bristles / hooks). A positive value indicates an overrepresentation of that appendage type in the cluster. *** P<=0.001; ** P<=0.01; * P<= 0.05; ns, not significant.*

Discriminant analysis (DA), based on the adhesivity data, separated the three seed clusters derived from the cluster analysis (Wilks' $\lambda = 0.308$; $\chi^2 = 70.6$, df = 14, $P<0.001$). The correspondence between the DA ordination plot based on the adhesivity scores (Fig. 4.2b) and the NMDS ordination plot based on the seed traits (Fig. 4.2a) illustrates this. The DA correctly classified 77.3% of the 66 seed species into the three seed clusters (Table 4.6). Most species of cluster SSA and SWA were correctly classified (75% and 89.2%, respectively), while only 33.3% of the species in cluster SUA was correctly classified. Wrongly assigned species of the clusters SUA and SSA were almost all assigned to cluster SWA, while wrongly assigned species of cluster SWA were mainly assigned to cluster SUA. This intermediate position of cluster SWA was not reflected in the cluster analysis, where cluster SWA was separated from both other clusters on a higher hierarchical level.

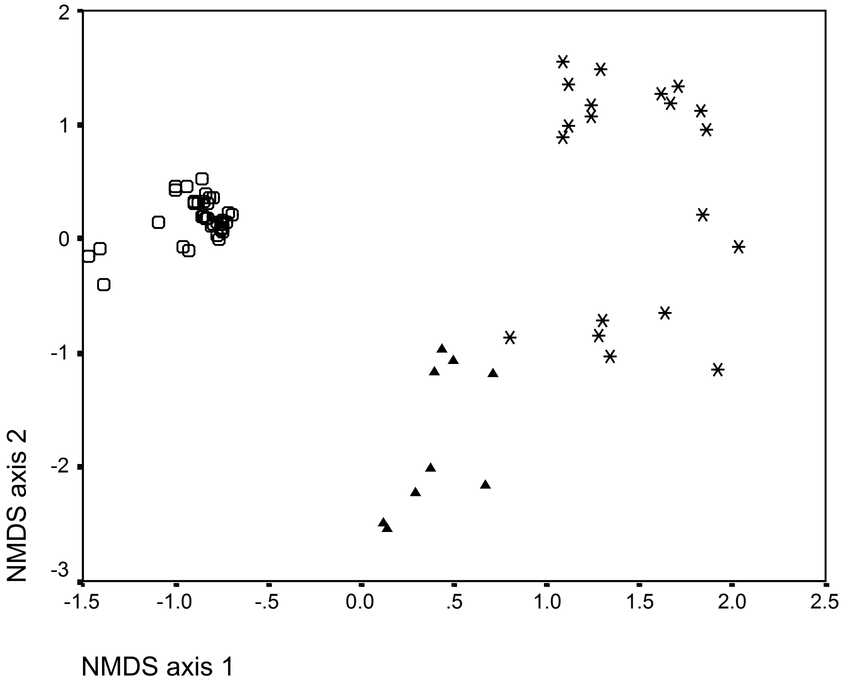
Table 4.5 *Overview of the adhesivity scores (AS) of the different seed clusters on the seven furs. (SUA, seeds with unspecialized appendages; SSA, seeds with specialized appendages; SWA, seeds without appendages; n = number of seed species in the clusters). A one-Way ANOVA was used to test the differences between the clusters. Values are group averages and groups that differ significantly are indicated with letters.*

	F	SUA	SSA	SWA
n		9	20	37
Horse	10.51***	0.04 a	0.29 b	0.08 a
Rabbit	17.14***	0.05 a	0.23 b	0.08 a
Holstein	9.04***	0.09 a	0.29 b	0.15 a
Roe deer	9.36***	0.14 a	0.41 b	0.21 a
Wild boar	5.40**	0.21 a	0.39 b	0.36 b
Sheep	19.58***	0.22 a	0.57 b	0.29 a
Galloway	16.86***	0.32 a	0.66 c	0.46 b
Global AS	15.80***	0.15 a	0.40 b	0.23 a

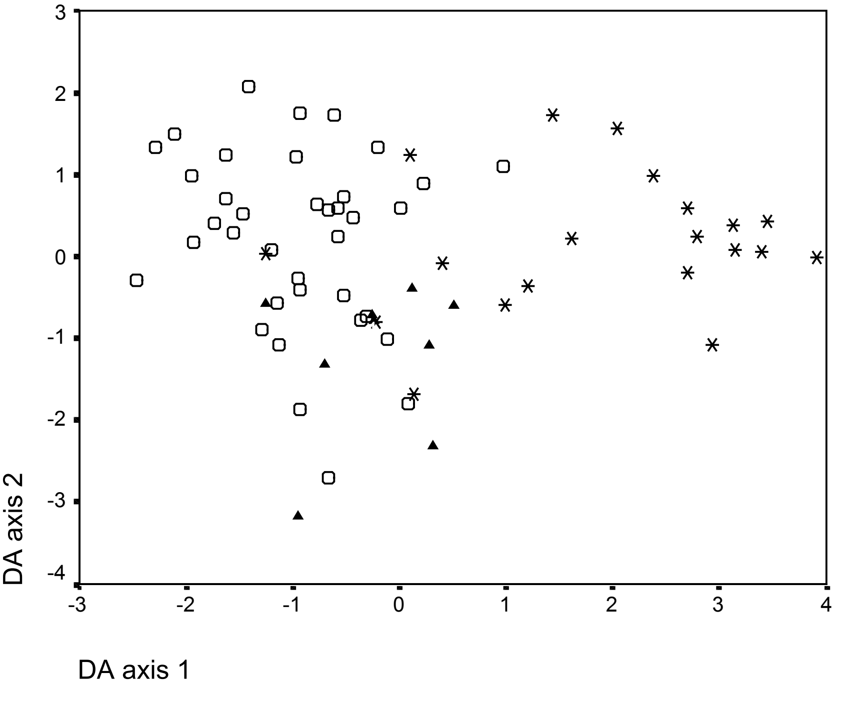
**** $P<=0.001$; ** $P<=0.01$; * $P<= 0.05$; ns, not significant.*

Table 4.6 *Crosstabulation of the number of seed species in the seed clusters (rows) and the predicted groups from the discriminant analysis (DA, columns). The last column shows the percentage of correctly classified species of each cluster* (Wilks' $\lambda = 0.308$; $\chi^2 = 70.6$, df = 14, $P<0.001$).

Cluster	DA group 1	DA group 2	DA group 3	total	% correctly assigned
SUA	3	0	6	9	33.3
SSA	1	15	4	20	75.0
SWA	3	1	33	37	89.2
total	7	16	43	66	77.3



(a)



(b)

Fig. 4.2 *Ordination of the 66 seed species by (a) nonmetric multidimensional scaling (NMDS) of the Gowers' proximity matrix, based on eight different seed traits, and (b) discriminant analysis (DA) based on adhesivity scores (86.9% of the total variance is explained by axis 1 and 13.1% by axis 2). The three seed type clusters are represented by different symbols: triangles, cluster SUA (seeds with unspecialized appendages); stars, cluster SSA (seeds with specialized appendages); circles, cluster SWA (seeds without appendages).*

Discussion

General

This study provides detailed experimental information on the adhesivity of a wide variety of seed types onto prepared furs of seven mammals. Our method allows species to be ranked on an ordinal scale, on the basis of their adhesivity scores. Both the 'global' adhesivity score (the average of the adhesivity scores on all tested furs) and the adhesivity scores on individual furs can be used for this purpose, since the ranking of the species showed relatively small differences between different furs. The adhesivity score, reflecting the epizoochorous dispersal potential, continually varies among species, ranging from extremely low to extremely high. In contrast to the formerly established idea that 'the' dispersal mechanism can be derived directly from the morphological properties of a seed (Grime et al. 1988, Hughes et al. 1994), it is thus likely that even species adapted to other dispersal modes or unspecialized species can disperse epizoochorously. Recently, species have been recognized to disperse in many different ways (Higgins et al. 2003b, Tackenberg et al. 2003). Questions regarding the relative efficiency of certain dispersal modes for a range of species are answerable after characterization and comparison of the relative dispersal potentials of the species on an ordinal scale, on the basis of indicator values of dispersal potential for different dispersal modes (Tackenberg et al. 2003). Efforts to establish a species ranking with respect to wind dispersal potential and, to a more limited extent, water dispersal potential have already been made by Tackenberg et al. (2003) and Lopez (2001), respectively. Our paper provides a method for species ranking in terms of epizoochorous dispersal potential.

Methodology

The adhesivity test method was chosen for its simplicity (e.g. in comparison with the method of Gorb & Gorb 2002), and preferred to other explored methods, such as one involving pushing the fur softly onto the seeds and then lifting it up. In the latter method, standardisation of the applied force onto the furs was complicated by the weight differences between the furs, and damage of fragile seeds (e.g. *Cirsium oleraceum)* was inevitable. Seed dropping onto the fur might have resulted in a somewhat conservative assessment of the adhesivity of seeds with adhesive adaptations, since the heaviest part of such seeds, which is usually not the most adhesive, touches the fur first when falling. In addition, the results may vary with intra-species variation in seed and fur characteristics (see also Kiviniemi 1996), which can depend on genetic factors, on the seed position in the inflorescence of a plant, on the season, or on the age, sex and health or body part of an animal (see also Agnew & Flux 1970, Mrotzek et al. 1999).

Possibilities abound for extending the test method used in this paper. For example, after the first rotation of the construction, followed by counting of the attached seeds, the construction could be tilted once again. This procedure could demonstrate that once seeds are attached to the fur, they are less likely to fall off rapidly. In other words, it would explain why the epizoochorous dispersal curve is very steep at the beginning, but exhibits a relatively fat tail, because once attached seeds tend to stay attached. Other variations of the tilt-method, such as shaking the fur construction, could imitate animal movement. In addition, the effect of weather conditions could be investigated by using dry, moist and wet furs. Special adaptations, such as mucus-secreting seed coats, could then be validated. For instance, *Plantago lanceolata* and *Prunella vulgaris* do not behave differently from other similarly shaped species in the present study, but might perform better on wet furs.

Fur traits and seed traits influencing adhesivity

The fur traits positively associated with high adhesivity scores were hair length, fur roughness, fur depth, hair undulation, and angle between hair and skin (Fig. 4.1). Galloway cattle, sheep and wild boar proved to be most suited for seed adherence, followed by roe deer, and finally Holstein cattle, rabbit and horse (Table 4.1). This is in agreement with observations of other authors who compared fur impact on seed adhesion, e.g. Heinken & Raudnitschka (2002) for wild boar and roe deer; Kiviniemi (1996) and Kiviniemi & Telenius (1998) for fallow deer and short-haired domestic cattle; Shmida & Ellner (1983) for sheep and goat.

The three seed clusters, based on eight seed traits (Table 4.4), had different adhesivity scores (Table 4.5). Cluster SSA -seeds with specialized appendages (awns, hairs, bristles, hooks)- scored significantly better than the two similarly scoring clusters SUA -seeds with unspecialized appendages (stems or remnants of the perianth)- and SWA (seeds without appendages). This was true for all furs except those of Galloway, on which cluster SWA had a score intermediate between SUA and SSA, and wild boar, where cluster SWA scored as high as SSA. Our results suggest that unspecialized appendages such as stems do not contribute to, or might even hamper seed adherence. However, the larger length of the seeds in cluster SUA in comparison with cluster SWA seeds may also explain their low adhesivity scores. Nevertheless, the seeds in cluster SSA, with specialized adhesive appendages such awns, hooks and bristles, adhered very well, although they were not differently sized from seeds in cluster SUA (Tables 4.3 and 4.4). The negative impact of seed length, seed width, seed thickness and seed weight on adhesivity was also higher if only the species without adhesive appendages were considered (Table 4.2). Our results suggest that specialized adhesive appendages can, at least partly, overcome the adhesive dispersal difficulties of larger-sized and heavy seeds (see also Kiviniemi & Telenius 1998). Only in the case of wild boar did the advantage of appendage-bearing seeds seemed to be absent (Table 4.5). Apparently, fur density and hair thickness have an opposite effect on certain seed types (interaction-effect of fur type and seed type) (Fig. 4.1). For instance, seeds with specialized adhesive appendages adhered better to the dense, thin-haired fur of sheep than on the thick-haired, not-dense fur of wild boar, while the reverse was true for seeds without appendages. In the fur of wild boar, many small seeds without appendages (e.g. *Lychnis flos-cuculi, Silene conica, Myosotis scorpioides, Lycopus europaeus, Glyceria maxima*) adhered better than seeds with very specialized adhesive appendages (e.g. *Torilis japonica, Bidens frondosa, Erodium cicutarium, Geum urbanum* and *Daucus carota*). The wild boars' thick hairs, implanted at low densities, apparently allow good penetration and retention of small unappendaged seeds, but offer relatively poor grip to seeds with sophisticated adhesive appendages (see also Mrotzek et al. 1999, and Heinken & Raudnitschka 2002). In contrast, the dense undulated fur of sheep, as well as the other furs, seemed better suited for attachment of seeds with specialized appendages.

The high degree of predictability of the adhesivity scores starting from the seed clusters (Fig. 4.2, Table 4.6) indicated that seed morphology is a good predictor for adhesivity on fur. Seeds with unspecialized appendages, however, behave more like unappendaged seeds than like seeds with specialized appendages (Fig. 4.2). Still, the predictive potential of seed morphological traits for adhesivity to fur does not imply that one seed morphological aspect determines 'the' seed dispersal mechanism of a plant. Wind-dispersal adaptations, for instance, also provide some seed adhesivity to fur. Unspecialized appendages do not appear to aid to seed adherence, and the functionality of specialized adhesive appendages depends on the fur type. In addition, it is important to realize that the adhesivity scores of the clusters

SWA and SUA are still about half those of cluster SSA. This suggests that it is likely that even seeds without epizoochorous adaptations can disperse successfully by epizoochory. The fact that even seeds with very low adhesivity scores (*Galeopsis tetrahit, Anthriscus sylvestris, Heracleum sphondylium, Angelica sylvestris, Alnus glutinosa*) have been observed to disperse epizoochorously in other studies (see App. 4.1), further adds to the evidence that many more species than previously thought occasionally disperse in the fur of mammals. Possibly, some of these species could adhere successfully by means of remains of other rough plant parts on the seeds.

However, full understanding of the process of epizoochory requires an integration of knowledge about all influencing factors. For instance, the probability of a seed to reach an animal's fur also depends on the abundance in the vegetation (Stender et al. 1997) and on plant traits such as the height of seed exposure (Fischer et al. 1996, Stender et al. 1997, Heinken 2000), the duration of the disseminating period (Fischer et al. 1996, Heinken 2000), the degree of seed exposure, and the ease of release from the parent plant. The latter occurs in the case of many wind-dispersed herbs in dry, sunny weather (Tackenberg et al. 2003) and might as such also influence epizoochory. Other key elements in epizoochorous dispersal are animal presence and behaviour (Fischer et al. 1996). Animal behaviour can also interact with seed traits. For instance, pronounced adhesive structures that promote seed attachment to fur may actually reduce the probability of successful dispersal by increasing grooming behaviour, especially if the seeds are accessible and noticeable to the animals (Sorensen 1986, Kiviniemi 1996).

Conclusions and consequences

The adhesivity scores, as a measure of epizoochorous seed dispersal potential, suggest that a continuum in adhesive capacity exists among all types of seeds and that for most species, seed morphology is probably not a limiting factor for epizoochorous dispersal. Species with high adhesivity scores probably have a higher potential to achieve long-distance dispersal through epizoochory. Although it remains arbitrary to define a treshhold in this context, we propose that species with a global adhesivity score higher than 0.3 attach well enough to fur to have a reasonable chance of being epizoochorously dispersed over long distances, at least if they are sufficiently abundant in the vegetation. Species such as *Juncus effusus, Eupatorium cannabinum, Torilis japonica* and *Urtica dioica*, for instance, with a global adhesivity score between 0.3 and 0.4, have been observed frequently in animal fur (App. 4.1).

Although we demonstrated the existence of an interaction-effect between seed morphology and fur type, which was best illustrated by comparing the fur of wild boar with other furs, our results indicate that specialized seed appendages, such as awns, hooks and bristles, considerably enhance adhesivity to fur. Seeds with unspecialized appendages, such as stems, did not perform better than unappendaged seeds.

Long-haired, deep, rough, undulating furs with a large angle between hairs and skin prooved to be most suited for dispersing seeds over large distances. This does not mean that animals with smooth, short-haired, shallow furs do not participate in epizoochorous seed dispersal. Smooth-furred small mammals such as rabbits and mice, for instance, probably contribute considerably to epizoochory on a local scale (see also Kiviniemi & Telenius 1998), especially in view of their high relative abundance in our landscapes. Domesticated large herbivores with smooth, straight, short hairs, such as horse and Holstein cattle are also expected to contribute to epizoochory on a rather local scale. Roe deer, with a somewhat longer and rougher fur, takes an intermediate position with respect to seed adhesivity, but might be of considerable importance, being the most abundant wild large mammal in the western European landscape. Also wild boar might be an important long-distance seed disperser, taking into account its high adhesivity scores and large home range (Briedermann 1990). The two furs with the highest adhesivity scores, however, belong to sheep and Galloway cattle. Therefore, the increasing importance of the latter animals in nature management seems a positive step from a plant dispersal point of view.

Acknowledgements

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App. 4.1 *Overview of the 66 study species, with a description of their diaspores (furher referred to as seeds) (column 2), seed traits (columns 3-10), seed cluster number (1=SUA; 2=SSA; 3=SWA) (column 11), adhesivity scores on the furs of seven herbivore species (column 12-18) and global adhesivity score (the average adhesivity of the seven furs) (column 19). Botanical nomenclature follows Lambinon et al. (1998). At least 38 of the plant species have been observed to disperse epizoochorously and at least 5 of the animal species have been reported as epizoochorous dispersal vectors (indicated by a reference number after the species name).*

		Seed traits									Adhesivity scores							
	diaspore description	appendage type	seed surface texture	appendage density	appendage length (mm)	seed length (mm)	seed width (mm)	seed thickness (mm)	seed weight (mg)	seed cluster	Horse[8]	Rabbit	Holstein cattle	Roe deer[7,8]	Wild boar[4,7]	Sheep[1,2]	Galloway cattle[3,9]	Mean adhesivity score
Achillea millefolium[7]	5	1	1	1	1.88	4.46	0.62	0.63	0.4	1	0.028	0.052	0.112	0.292	0.372	0.360	0.448	0.238
Agrostis canina[3]	3	2	1	1	1.54	2.09	0.31	0.31	0.02	2	0.424	0.628	0.476	0.720	0.584	0.724	0.848	0.629
Alisma plantago-aquatica	2	0	1	0	0	1.69	0.93	0.20	0.2	3	0.056	0.024	0.232	0.200	0.436	0.360	0.576	0.269
Alnus glutinosa[78]	2	0	3	0	0	2.85	2.15	0.82	1.2	3	0.016	0.020	0.040	0.064	0.264	0.200	0.340	0.135
Alopecurus pratensis[245]	3	2	4	1	4.31	4.79	1.80	0.76	0.4	2	0.356	0.440	0.308	0.660	0.496	0.724	0.784	0.538
Angelica sylvestris[2]	2	0	3	0	0	4.68	3.40	0.91	2	3	0.036	0.016	0.240	0.044	0.144	0.124	0.256	0.123
Anthoxanthum odoratum[23]	3	2	4	2	7.72	3.17	1.12	1.00	0.6	2	0.276	0.416	0.324	0.436	0.596	0.744	0.844	0.519
Anthriscus sylvestris[28]	2	0	1	0	0	7.43	1.08	1.08	5.2	3	0.008	0.004	0.012	0.004	0.188	0.136	0.272	0.089
Arrhenatherum elatius[28]	3	2	4	1	4.29	7.90	1.50	1.50	1.8	2	0.268	0.288	0.312	0.344	0.364	0.676	0.780	0.433
Bellis perennis[3]	2	0	4	0	0	1.40	0.64	0.11	0.02	3	0.360	0.112	0.432	0.624	0.488	0.448	0.616	0.440
Bidens frondosa[8]	2	5	5	2	3.12	7.23	2.17	0.68	3	2	0.288	0.336	0.400	0.356	0.340	0.716	0.716	0.450
Bromus sterilis[7]	3	5	5	1	24.23	16.05	1.28	1.28	8.75	2	0.080	0.432	0.116	0.444	0.528	0.788	0.876	0.466
Caltha palustris	1	0	1	0	0	2.65	1.22	1.22	1.9	3	0.000	0.006	0.013	0.031	0.200	0.088	0.344	0.097
Capsella bursa-pastoris[2347]	1	0	1	0	0	0.87	0.44	0.44	0.1	3	0.260	0.472	0.308	0.552	0.448	0.420	0.548	0.430
Centaurea jacea[24]	2	0	1	0	0	3.29	1.25	1.25	2.3	3	0.004	0.024	0.028	0.072	0.320	0.148	0.352	0.135
Centaurea nigra[8]	4	3	1	2	0.35	3.51	1.27	1.27	2.4	2	0.000	0.036	0.200	0.080	0.304	0.240	0.288	0.164
Chelidonium majus	1	0	1	0	0	1.30	0.77	0.77	0.6	3	0.012	0.088	0.084	0.260	0.420	0.232	0.412	0.215
Cirsium oleraceum	4	3	1	3	15.92	4.31	1.53	0.96	4.5	2	0.020	0.000	0.020	0.040	0.000	0.000	0.280	0.051
Crepis biennis	4	3	2	3	5.76	4.70	0.60	0.60	1.4	2	0.060	0.124	0.148	0.176	0.436	0.444	0.644	0.290
Daucus carota[124]	2	4	5	3	1.00	2.78	1.30	0.78	0.8	2	0.228	0.340	0.428	0.404	0.444	0.748	0.732	0.475
Erodium cicutarium	2	5	5	1	7.85	4.81	0.91	0.91	1	2	0.464	0.272	0.488	0.528	0.424	0.640	0.576	0.485
Eupatorium cannabinum[38]	4	3	2	3	4.26	2.66	0.33	0.33	0.4	2	0.208	0.124	0.312	0.544	0.272	0.304	0.528	0.327
Fallopia japonica	6	1	2	1	3.01	9.92	4.87	4.87	2.2	1	0.060	0.016	0.072	0.016	0.104	0.112	0.348	0.104
Festuca pratensis[38]	3	0	1	0	0	6.38	1.34	0.77	2.4	3	0.040	0.012	0.020	0.136	0.376	0.228	0.520	0.190
Filipendula ulmaria[4]	2	0	2	0	0	3.01	1.23	0.58	0.8	3	0.008	0.016	0.128	0.140	0.436	0.216	0.500	0.206
Fraxinus excelsior	2	1	2	1	2.44	42.35	8.51	0.37	59.2	1	0.000	0.000	0.000	0.000	0.008	0.008	0.004	0.003
Galeopsis tetrahit[4]	2	0	1	0	0	3.17	2.20	1.66	5.5	3	0.000	0.000	0.000	0.044	0.156	0.022	0.267	0.070
Galinsoga quadriradiata[8]	4	3	4	3	1.04	2.40	0.40	0.40	0.2	2	0.356	0.536	0.496	0.612	0.524	0.756	0.772	0.579
Geum urbanum[245678]	2	5	4	1	3.16	3.76	1.36	0.72	1.5	2	0.236	0.372	0.232	0.432	0.436	0.688	0.784	0.454
Glyceria fluitans[38]	3	0	2	0	0	5.71	1.05	1.05	0.6	3	0.056	0.060	0.112	0.100	0.296	0.344	0.680	0.235

		Seed traits									Adhesivity scores							
	diaspore description	appendage type	seed surface texture	appendage density	appendage length (mm)	seed length (mm)	seed width (mm)	seed thickness (mm)	seed weight (mg)	seed cluster	Horse[8]	Rabbit	Holstein cattle	Roe deer[7,8]	Wild boar[4,7]	Sheep[1,2]	Galloway cattle[3,9]	Mean adhesivity score
Glyceria maxima	3	0	2	0	0	3.56	0.48	0.76	0.2	3	0.096	0.192	0.108	0.268	0.500	0.336	0.492	0.285
Heracleum sphondylium[2]	2	0	2	0	0	9.47	7.28	0.34	3.8	3	0.048	0.040	0.040	0.088	0.152	0.148	0.196	0.102
Juncus effusus[348]	7	1	3	1	3.13	1.91	1.25	1.25	0.4	1	0.212	0.160	0.172	0.368	0.348	0.420	0.536	0.317
Knautia arvensis	2	3	4	3	1.12	4.83	2.41	1.49	5.6	2	0.000	0.008	0.004	0.052	0.144	0.092	0.336	0.091
Leucanthemum vulgare[2]	2	0	3	0	0	2.46	0.78	0.78	0.8	3	0.076	0.040	0.140	0.064	0.476	0.344	0.504	0.235
Lychnis flos-cuculi	1	0	2	0	0	0.55	0.55	0.55	0.1	3	0.204	0.168	0.360	0.476	0.536	0.424	0.652	0.403
Lycopus europaeus[48]	2	0	2	0	0	1.43	1.04	0.48	0.4	3	0.072	0.152	0.208	0.224	0.512	0.412	0.504	0.298
Lysimachia vulgaris	1	0	2	0	0	1.71	1.05	1.05	0.4	3	0.028	0.032	0.060	0.200	0.396	0.288	0.564	0.224
Lythrum salicaria[8]	1	0	1	0	0	0.90	0.28	0.28	0.02	3	0.364	0.188	0.472	0.580	0.456	0.472	0.644	0.454
Myosotis scorpioides	2	0	2	0	0	0.87	0.87	0.59	0.2	3	0.160	0.144	0.168	0.384	0.512	0.368	0.540	0.325
Myosoton aquaticum	1	0	1	0	0	1.61	1.13	1.13	0.5	3	0.030	0.050	0.120	0.230	0.340	0.230	0.510	0.216
Myrrhis odorata	2	0	3	0	0	18.15	2.42	2.42	25	3	0.000	0.000	0.000	0.000	0.028	0.032	0.084	0.021
Oenothera biennis	1	0	2	0	0	1.67	1.02	1.02	0.6	3	0.028	0.064	0.040	0.176	0.428	0.300	0.456	0.213
Papaver rhoeas[24]	1	0	2	0	0	0.54	0.54	0.54	0.02	3	0.256	0.056	0.484	0.552	0.492	0.464	0.604	0.415
Phalaris arundinacea[8]	3	3	1	2	1.05	3.11	0.93	0.93	0.2	2	0.172	0.180	0.200	0.468	0.480	0.520	0.620	0.377
Phragmites australis	3	3	1	3	5.88	2.95	0.78	0.78	0.2	2	0.456	0.152	0.404	0.564	0.448	0.616	0.760	0.486
Plantago lanceolata[38]	1	0	1	0	0	2.61	1.19	1.19	1.8	3	0.000	0.004	0.240	0.084	0.332	0.328	0.404	0.199
Polygonum hydropiper	2	0	2	0	0	3.56	1.85	1.25	2.2	3	0.004	0.020	0.016	0.040	0.220	0.212	0.364	0.125
Prunella vulgaris[4]	2	0	1	0	0	1.64	0.91	0.91	0.6	3	0.068	0.032	0.036	0.164	0.472	0.340	0.392	0.215
Pulicaria dysenterica	4	3	1	3	3.27	1.47	0.22	0.22	0.1	2	0.400	0.540	0.444	0.588	0.332	0.672	0.752	0.533
Ranunculus acris	2	1	2	1	0.42	3.62	2.40	1.02	2.4	1	0.000	0.000	0.016	0.060	0.236	0.116	0.336	0.109
Rhinanthus minor	1	0	2	0	0	4.59	3.56	0.63	3.2	3	0.004	0.008	0.004	0.020	0.204	0.064	0.376	0.097
Rumex hydrolapathum	6	1	2	1	3.48	4.99	3.84	3.84	5.8	1	0.008	0.020	0.136	0.032	0.124	0.128	0.188	0.091
Rumex obtusifolius[258]	6	1	3	1	3.98	4.42	3.50	3.50	3	1	0.036	0.088	0.212	0.252	0.272	0.348	0.348	0.222
Scrophularia nodosa[4]	1	0	2	0	0	0.77	0.39	0.39	0.2	3	0.232	0.200	0.372	0.444	0.500	0.428	0.648	0.403
Scutellaria galericulata	2	0	3	0	0	1.38	1.38	1.38	0.6	3	0.016	0.060	0.092	0.248	0.368	0.396	0.412	0.227
Senecio jacobea[8]	4	3	4	3	4.36	1.74	0.49	0.49	0.3	2	0.160	0.240	0.300	0.432	0.332	0.640	0.604	0.387
Silene conica	1	0	2	0	0	0.93	0.74	0.74	0.4	3	0.068	0.072	0.204	0.264	0.520	0.356	0.652	0.305
Silene dioica[4]	1	0	2	0	0	1.12	1.12	1.12	0.8	3	0.004	0.076	0.272	0.124	0.400	0.416	0.412	0.243
Sparganium erectum	5	1	2	1	3.39	7.19	3.08	3.08	14.6	1	0.004	0.000	0.004	0.016	0.084	0.172	0.252	0.076
Symphytum officinale	2	0	1	0	0	4.22	2.60	2.60	7.2	3	0.000	0.004	0.000	0.012	0.044	0.056	0.224	0.049
Tanacetum vulgare[348]	5	1	2	1	1.89	4.16	0.74	0.74	0.6	1	0.036	0.108	0.100	0.252	0.328	0.332	0.464	0.231
Torilis japonica[24]	2	4	5	3	0.12	3.15	1.66	1.18	2	2	0.076	0.304	0.196	0.240	0.332	0.628	0.692	0.353
Trifolium dubium	1	0	2	0	0	2.48	2.11	1.08	2	3	0.027	0.093	0.040	0.053	0.347	0.387	0.400	0.192
Urtica dioica[24678]	2	0	3	0	0	1.85	1.14	0.71	0.3	3	0.176	0.288	0.176	0.316	0.552	0.556	0.684	0.393
Verbascum thapsus	1	0	2	0	0	0.72	0.38	0.38	0.2	3	0.136	0.144	0.280	0.496	0.488	0.516	0.460	0.360

Table legend: see next page.

Legend App. 4.1

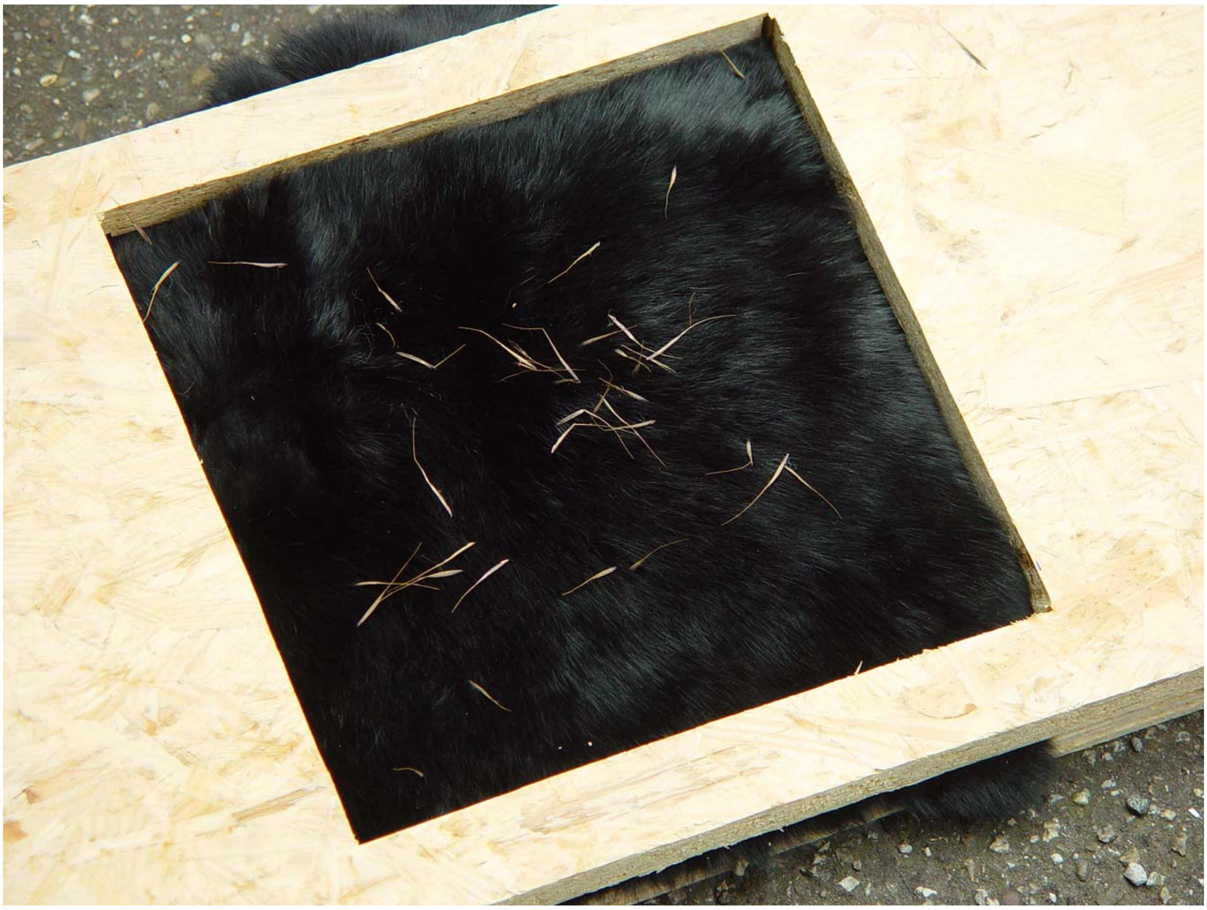
[1]Shmida & Ellner (1983); [2]Fischer et al. (1996); [3]Stender et al. (1997); [4]Mrotzek et al. (1999); [5]Heinken (2000); [6]Graae (2002); [7]Heinken & Raudnitschka (2002); [8]Schmidt et al. (2005) [9]Chapter 2. Diaspore description: (1) seed, (2) fruit, (3) fruit with bracts, (4) fruit with pappus, (5) fruit with remnants of perianth, (6) fruit with stem and remnants of perianth, (7) capsule with stem and remnants of perianth. Appendage type: (0) no appendages, (1) stem or remnants of perianth, (2) awn, (3) hairs, (4) bristles, (5) hooks. Surface texture: (1) smooth, (2) slightly rough, (3) rough, (4) hairy, (5) sticky; Appendage density: (0) no appendage, (1) one appendage, (2) low density (2-50), (3) high density (>50).

App. 4.2 *Overview of the fur traits of the different fur samples.*

	hair length (mm)	fur depth (mm)	hair thickness (rank)	fur density (rank)	fur roughness (rank)	hair undulation (rank)	hair-skin angle (rank)
Horse	15	2	3	3	2	1	1
Rabbit	32	13	2	4	1	1	2
Holstein cattle	20	2	3	2	2	1	1
Roe deer	55	15	4	4	4	2	2
Wild boar	100	25	5	1	5	1	3
Sheep	80	40	1	5	6	4	5
Galloway cattle	70	20	3	2	3	3	4

App. 4.3 *Experimental construction for the seed adhesivity tests.*

(see next page)










Chapter 5

Experimental assessment of plant seed retention times in fur of cattle and horse

Abstract

Epizoochorous dispersal of plant seeds is an important long-distance dispersal mechanism. Yet little is known about retention times of seeds in animal furs and hence about potential dispersal distances of the seeds. Here, we used marked seeds of 12 plant species to determine seed depletion curves on Galloway cattle and Haflinger horse in three vegetation types (forest, tall herbage vegetation and meadow), in both dry and rainy weather conditions. In the long fur of Galloway cattle, seeds were retained significantly longer than in the short fur of Haflinger horse. In general, seed retention times were not considerably affected by the structure of the surrounding vegetation. The impact of the weather was negligible, only affecting the retention of some plant species. Negative exponential functions were fitted to the seed depletion curves. Using the parameters of curve estimations in the different conditions of animal species and vegetation structure, half-life seed retention times of up to 13 hours for Galloway cattle and up to more than 4 hours for Haflinger horse could be calculated, with corresponding potential half-life dispersal distances in the order of magnitude of tens of metres to a few kilometres. Different seed traits correlated with seed retention times in the long cattle fur and in the short horse fur, respectively.

Keywords

Epizoochory, fur, Galloway cattle, Haflinger horse, long-distance seed dispersal, seed traits

Introduction

Seed dispersal has become a key issue in plant ecology. It enables plant populations to maintain sufficient genetic diversity and to colonize new habitats, both on a local and on a landscape scale (Bullock et al. 2002). However, large-scale destructive human activities have globally led to severe habitat loss, degradation and fragmentation. The remaining suitable habitat patches for wild plant populations have become small and spatially isolated, and seed dispersal has developed into a serious bottleneck for genetic exchange and colonization (e.g. Turnbull et al. 2000, Zobel et al. 2000, Verheyen et al. 2003a, b). As a consequence, the fitness and long-term survival of many plant (meta)populations is threatened. Since most natural processes and traditional farming practises effecting long-distance seed dispersal are severely restricted or even absent in the present cultivated landscapes (Bonn & Poschlod 1998, Poschlod & Bonn 1998, Bruun & Fritsbøger 2002), the restoration or maintenance of the remaining long-distance seed dispersal mechanisms may be crucial for the long-term survival of plant populations.

Epizoochory or the external transport of plant seeds by animals is one of the most effective long-distance dispersal mechanisms (e.g. Fischer et al. 1996, Stender et al. 1997, Mrotzek et al. 1999). Nevertheless, zoochory is a still largely neglected aspect of seed ecology. Levin et al. (2003) therefore emphasized the requirement of high-quality data on the operative factors of zoochorous seed dispersal, to enable parameter estimation for dispersal modelling. The importance of the identification and characterization of long-distance dispersal pathways has also been stressed by Higgins & Richardson (1999), who showed that only a small proportion (0.1%) of seeds moving long distances (1-10 km) can lead to an order of magnitude increase in predicted spread rate.

Apart from predominantly descriptive studies on epizoochory (e.g. Agnew & Flux 1970, Lacey 1981, Fischer et al. 1996, Stender et al. 1997, Mrotzek et al. 1999, Heinken 2000, Heinken et al. 2001, Heinken & Raudnitschka 2002, chapters 2 and 3), a number of papers focus on the experimental investigation of seed dispersal. To assess epizoochorous retention times of seeds, they used either cotton clothes (Bullock & Primack 1977) or prepared furs (Fischer et al. 1996, chapter 4) as substitutes for animals, or they used live rodents in laboratory conditions (Sorensen 1986, Kiviniemi & Telenius 1998) or large mammals in field conditions (e.g. Shmida & Ellner 1983, Fischer et al. 1996, Kiviniemi 1996, Kiviniemi & Eriksson 1999, Graae 2002). In these studies, experimental retention curves of marked seeds suggested dispersal distances in the order of magnitude of metres for small mammals such as hare (Sorensen 1986) and wood mouse (Kiviniemi & Telenius 1998), hundreds of metres for larger mammals such as fallow deer (Kiviniemi 1996) and short-furred cattle (Kiviniemi 1996, Kiviniemi & Eriksson 1999), and up to several kilometres for sheep (Shmida & Ellner 1983, Fischer et al. 1996). Some of the mentioned studies focused on specific aspects of epizoochory, e.g. the influence of adhesive appendages on seed retention in fur (Kiviniemi & Telenius 1998), the influence of seed morphology, number and location on seed removal behaviour by the animal vector (Sorensen 1986, Kiviniemi 1996), or the difference between animal species (Shmida & Ellner 1983, Kiviniemi 1996). Many factors influencing seed retention, however, remain uninvestigated. The role of the surrounding vegetation, for instance, has solely been studied by Bullock & Primack (1977), using cotton covered boards in uniform vegetation stands of three herbaceous plants. Also the influence of the weather conditions has only been briefly been documented by Graae (2002). As a consequence, much remains to be elucidated.

In this study, we investigate the retention behaviour of 12 seed species with different morphological characteristics in the fur of two species of large herbivores, in three vegetation types and two weather conditions. The following specific research hypotheses are adressed:

- Seeds are longer retained on long-furred cattle than on short-furred horse.
- Seed retention times are higher in rainy weather circumstances.
- Seed detachment is faster in tall-growing vegetations than in short vegetations.
- Small seeds and seeds with adhesive adaptations are retained longer in fur.

Material and methods

Study site

The retention experiments were conducted in September 2001, in the 100 ha nature reserve 'Mechels Broek' (Mechelen, Belgium), a brook valley system mainly consisting of wet to moist riverine grasslands, alternating with tall herbage vegetations and forest patches.

Experimental design

Diaspores (further referred to as seeds) of 12 native plant species (see Table 5.1) -chosen for their contrasting diaspore morphology- were obtained from Ecoflora (Halle, Belgium), a company selling organically grown native plant material. Of each species, eight relevant seed traits were measured (see Table 5.1). The seeds were marked with fluorescent tree marker dye, and seed mixtures were composed of a fixed number of seeds for each species (see Table 5.1). The mixtures contained larger numbers of the smaller seeds, and smaller numbers of the larger seeds, reflecting the ecological trade-off between seed size and seed number, and at the same time minimizing the chance of grooming behaviour by the animals, which is induced by large seeds (see Sorensen 1986, Kiviniemi 1996).

Galloway cattle and Haflinger horse (shoulder-height: 125 cm and 143 cm, respectively; ventral height: 42 cm and 58 cm, respectively) were selected as representative herbivores used for grazing projects with robust breeds of large herbivores, such as in many Flemish (Eggermont et al. 1996) and European nature reserves. The hairs of the Galloway fur were approximately 10 cm long, undulated and implanted at a large angle with the skin, while the hairs of the horse fur were approximately 3 cm long, straight, and implanted at a very small angle with the skin.

The three most relevant vegetation types in the study area were selected, reflecting three main land use forms resulting from different management: forest, tall herbage vegetation and meadow. The forest was an *Alnus glutinosa* stand (trees of about 5 m high), where the body of the experimental animals was almost constantly in contact with tree branches and up to 2 m tall stems of understory plants such as *Bidens tripartita, Carex acutiformis, Glyceria maxima, Juncus effusus, Lycopus europaeus, Rubus* spp., *Symphytum officinale* and *Urtica dioica*. In the tall herbage vegetation, the body of the animals made contact with the stems of the up to 2 metres high herbaceous vegetation (dominated by *Glyceria maxima, Juncus* *effusus, Lycopus europaeus, Lythrum salicaria, Phalaris arundinacea* and *Rumex hydrolapathum*), and only occasionally with shrubs or branches of a young tree. In the meadow, the recently mown vegetation of less than 20 cm height (dominated by *Agrostis capillaris, Holcus lanatus, Juncus effusus, Phalaris arundinacea, Plantago lanceolata, Polygonum amphibium* and *Ranunculus repens*) made only contact with the animals' legs.

Table 5.1 *Species used in the retention experiments, composition of the experimental seed mixtures, and eight relevant seed traits.*

species	seeds in mixture	appendage type (categorical)	seed surface texture (ordinal)	appendage number (ordinal)	appendage length (mm)	seed length (mm)	seed width (mm)	seed thickness (mm)	seed weight (mg)
Agrimonia eupatoria[1, 2, 3]	7	2	3	3	1.63	5.69	4.84	4.84	35.00
Anemone nemorosa	40	2	2	1	0.78	3.42	1.69	1.12	2.40
Centaurea jacea	40	0	1	0	0.00	2.87	1.20	0.92	2.00
Cynoglossum officinale	15	0	3	0	0.00	5.97	4.70	1.89	23.00
Daucus carota[2]	50	2	3	2	1.00	2.58	1.36	0.75	1.20
Galium odoratum[4]	7	0	3	0	0.00	2.21	2.21	1.65	6.00
Geum urbanum[4]	40	2	2	1	3.98	3.66	1.35	0.70	2.00
Heracleum sphondylium	15	0	2	0	0.00	7.71	5.42	0.29	6.60
Knautia arvensis	15	1	2	3	1.15	4.90	1.98	1.41	4.50
Oenothera biennis	50	0	2	0	0.00	1.62	0.90	0.93	0.40
Prunella vulgaris	50	0	1	0	0.00	1.82	0.89	0.69	0.60
Ranunculus acris[2, 3]	50	2	2	1	0.40	2.69	2.03	1.00	1.40

Type of adhesive appendage: (0) no appendages, (1) hairs, (2) bristles or hooks; Seed surface texture rank: (1) smooth, (2) slightly rough, (3) rough; Rank of appendage number: (0) no appendages, (1) 1 appendage, (2) low appendage number (2-50), (3) high appendage number (>50). Appendage length, seed length, seed width and seed thickness were measured as the average of 15 randomly chosen seeds, while seed weight was calculated by dividing the weight of 50 seeds by 50. Species with superscript numbers: used in similar retention experiments by [1]*Kiviniemi (1996);* [2]*Kiviniemi & Telenius (1998);* [3]*Kiviniemi & Eriksson (1999);* [4]*Graae (2002). Nomenclature follows Lambinon et al. (1998).*

For the experimental observation of epizoochorous seed retention, one tame individual of each herbivore species, representative in terms of fur properties and behaviour, was -for practical reasons- used repeatedly. To imitate the effect of moderately soft rain, one randomly chosen side of each animal was softly sprayed with about 0.5 l water at the start of each observation series, until the fur was moist but not soaked (approximating observed fur conditions after moderate rainfall). Subsequently, a seed mixture was applied to the back and upper flank -the most important body parts in terms of long-distance seed dispersal- of the dry respectively the wet side of the animal, gently by hand and always by the same person. During the following 20 hours, the seed numbers at the dry respectively the wet side of the animal were monitored regularly, using the following protocol. During the first 30 minutes, the animal walked a distance of 100 m on a lead, after which the seeds were counted (census time 1). This procedure was repeated in the following 30 minutes (census time 2). Then, the animal was left alone for 45 minutes, after which the seeds were counted again (census time 3). Subsequently, another distance of 300 m was walked on a lead, followed by seed counting (census time 4). Finally, the animal was allowed to move freely during the night, after which the seeds were counted a last time (census time 5).

For each animal species, this procedure was performed three times (on three different days with comparable ‘background’ weather conditions) in each vegetation type. The dataset thus consisted of 18 walks and 36 observations (3 walking days x 2 animal species x 3 vegetation types x 2 weather conditions).

Data analysis

The seeds counted on all count-occasions were standardized to percentages of the initial number of applied seeds. The data were analysed using SPSS 10.0 statistical software (SPSS 1999), unless mentioned otherwise. Non-parametric statistics were used only if the assumptions of normality and equality of variance were not fulfilled. The graphs were produced using Sigmaplot 5.0.

First, the effects of animal species and weather condition on the number of seeds were analysed by means of the MANOVA approach for analysing repeated measures designs (O’Brien & Kaiser 1985). In our model, animal species and weather conditions are between-subjects effects, whereas the census-time is the within-subjects effect. The walking day is the block-factor. Separate analyses were performed for each plant species and vegetation type.

Secondly, the data were presented in seed depletion curves, with time units on the x-axis. To reveal the underlying mathematical dispersal function of the seed depletion curves, regression analysis was applied using the program Sigmaplot 5.0. Several functions were fitted to the data (linear-linear, exponential-linear, log-linear, log-exponential), but the following function fitted the data best: proportion of seeds remaining attached after adhesion (which occurs at t=0) = exp (-b x t). With the help of the parameter b, the half-life retention time $t_{½}$= (ln2) / b, i.e. the time at which 50% of the initial seed number was detached, was calculated for each seed species in the different conditions of herbivore species and vegetation structure. Since the two weather conditions did not differ significantly, averages of both weather conditions were used. Using a Friedman test with multiple comparisons (Siegel & Castellan 1988), the half-life retention times were tested for significant differences between the three vegetation types (for each animal species separately).

Finally, to reveal the relationship between seed retention and seed morphology, the mean half-life retention times of the experimental plant species, in the different conditions of animal species and vegetation type, were correlated with their quantitative seed traits (Table 5.1), using the Spearman rank correlation coefficient (Siegel & Castellan 1988).

Results

Role of animal species and weather in seed depletion

For most species, seed numbers decreased significantly in all conditions in the course of the 20-hours observation series (Fig. 5.1, Table 5.2). The effect of animal species was in general significant, with more seeds remaining on Galloway cattle than on Haflinger horse. Some species deviated somewhat from this trend, e.g. the relatively small and light seeds (*Geum urbanum*, *Ranunculus acris, Prunella vulgaris, Centaurea jacea* and *Oenothera biennis)* were not always significantly better retained on Galloway than on horse (Table 5.2). The difference in weather conditions did in general not affect the seed numbers. An interaction effect between time and animal existed for some species, while an interaction effect between time and weather occurred only exceptionally.

Table 5.2 *Effects of time, animal species and weather condition on the epizoochorous seed retention of 12 plant species in three vegetation types (GLM, repeated measures).*

plant species	vegetation type	test of between-subjects effects			multivariate tests of within-subjects effects			
		animal	weather	block factor	time	time*animal	time*weather	time*block factor
		df=1	df=1	df=2				
Agrimonia eupatoria	forest	42.4***	1.0ns	0.2ns	41.3**	8.5ns	0.3ns	0.9ns
	tall herbage	61.9***	3.5ns	1.3ns	1266.3***	5.5ns	5.2ns	1.3ns
	meadow	84.6***	0.8ns	0.1ns	71.2ns	17.3ns	0.7ns	0.7ns
Anemone nemorosa	forest	22.8**	0.2ns	0.33ns	147.1**	9.8*	1.8ns	0.6ns
	tall herbage	12.8**	0.3ns	2.1ns	1251.6***	36.5**	33.9**	8.1**
	meadow	35.7**	0.2ns	0.7ns	20.9ns	8.4ns	0.7ns	0.9ns
Centaurea jacea	forest	27.6**	2.6ns	3.1ns	400.0***	4.8ns	3.8ns	1.2ns
	tall herbage	0.1ns	0.6ns	0.1ns	502.8***	3.3ns	0.9ns	1.6ns
	meadow	24.3**	0.01ns	0.62ns	96.7 ns	1.6ns	1.1ns	1.8ns
Cynoglossum officinale	forest	75.6***	0.1ns	0.6ns	1432.6***	10.0*	5.6ns	1.3ns
	tall herbage	13.4**	3.7ns	0.4ns	122.8**	3.8ns	1.8ns	1.3ns
	meadow	52.7**	0.01ns	0.5ns	109.2ns	15.0ns	0.1ns	0.8ns
Daucus carota	forest	11.3*	2.3ns	2.7ns	2373.3***	10.1*	2.1ns	1.2ns
	tall herbage	7.6*	8.1*	6.8*	342.3***	4.3ns	1.3ns	1.3ns
	meadow	17.3**	0.2ns	1.3ns	252.4*	32.4ns	6.3ns	0.8ns
Galium odoratum	forest	59.1***	0.1ns	0.8ns	95.1**	9.0*	0.5ns	0.6ns
	tall herbage	36.9**	0.3ns	5.0*	766.8***	9.4*	0.96ns	0.9ns
	meadow	131.3***	0.00ns	2.2ns	660.6**	585.3**	0.8ns	6.8*
Geum urbanum	forest	22.9***	5.4ns	1.1ns	588.5***	58.9**	3.4ns	5.5*
	tall herbage	0.1ns	0.01ns	4.1ns	199.4**	3.1ns	0.4ns	0.9ns
	meadow	38.8**	1.5ns	2.4ns	290.0*	67.7ns	1.2ns	1.7ns
Heracleum sphondylium	forest	198.6***	41.2***	5.0*	449.1***	152.5**	21.6*	3.3ns
	tall herbage	2.4ns	7.3*	8.5*	425.0***	1.8ns	2.2ns	1.5ns
	meadow	7.6*	0.4ns	0.4ns	20743.3**	6.4ns	234.5*	1.1ns
Knautia arvensis	forest	43.0***	0.8ns	0.8ns	68.5**	14.4*	0.8ns	1.1ns
	tall herbage	5.7*	0.01ns	0.7ns	243.8***	12.7*	0.2ns	1.0ns
	meadow	26.3**	0.2ns	1.0ns	240.6*	459.6*	0.9ns	1.5ns
Oenothera biennis	forest	6.9*	2.8ns	2.0ns	740***	6.9ns	3.9ns	0.7ns
	tall herbage	3.5ns	2.3ns	4.2ns	437.6***	4.6ns	16.6*	1.6ns
	meadow	12.9*	0.02ns	0.2ns	2336.5*	54.1ns	5.5ns	1.9ns
Prunella vulgaris	forest	2.3ns	3.4ns	3.1ns	1950.2***	1.7ns	2.3ns	1.6ns
	tall herbage	6.3*	0.00ns	7.7*	767.4***	14.1*	1.2ns	1.9ns
	meadow	3.3ns	2.3ns	3.5ns	59.6ns	8.1ns	35.8ns	0.9ns
Ranunculus acris	forest	3.4ns	3.6ns	0.3ns	659.3***	16.2*	5.7ns	1.5ns
	tall herbage	0.2ns	3.3ns	5.5*	410.5***	5.1ns	1.7ns	1.6ns
	meadow	13.9*	0.3ns	0.7ns	150.4ns	22.6ns	1.7ns	1.0ns

*The multivariate tests are based on Pillai's test statistic. *** P<=0.001; ** P<=0.01; * P<= 0.05; ns, not significant.*

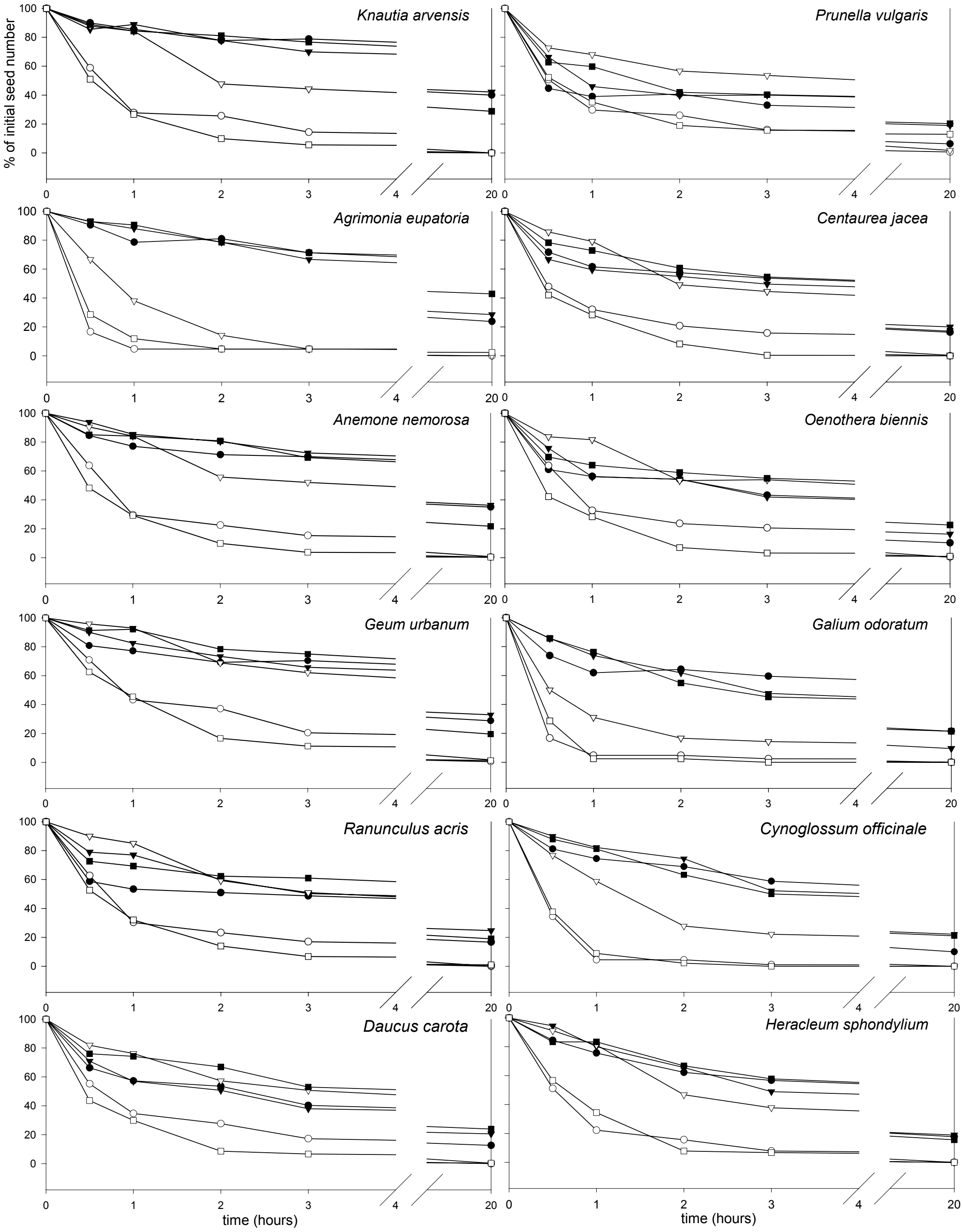


Fig. 5.1 *Seed depletion curves of 12 species in different conditions (filled symbols, Galloway cattle; empty symbols, Haflinger horse; circles, forest; triangles, tall herbage vegetation; squares, meadow). Values represent averages of the three replicates and the two weather conditions.*

Half-life seed retention times and role of vegetation type

The seed depletion curves (Fig. 5.1) had a negative exponential form and were comparable for all 12 seed species. Curve fitting of the seed depletion curves resulted in the parameters shown in App. 5.1. The calculated half-life seed retention times were higher on Galloway cattle than on Haflinger horse, but only on horse they differed significantly between the different vegetation structures (Friedman tests, see Table 5.3).

Table 5.3 *Half-life retention times ($t_{1/2}$, in hours) of 12 seed species on Galloway cattle and on Haflinger horse, in three vegetation types (average of two weather conditions). $T_{1/2}$ was calculated using the parameters resulting from the fitting of an exponential function on the seed depletion data (see App. 5.1). A Friedman test was used to compare the mean $t_{1/2}$ in the three vegetation types within each herbivore species.*

	Galloway cattle				Haflinger horse			
	forest	tall herbage	meadow	all vegetations	forest	tall herbage	meadow	all vegetations
Agrimonia eupatoria	7.37	8.06	12.84	9.12	0.20	0.75	0.29	0.38
Anemone nemorosa	8.35	10.83	6.86	8.56	0.75	2.96	0.53	1.09
Centaurea jacea	2.42	2.13	2.91	2.47	0.64	2.37	0.47	0.95
Cynoglossum officinale	3.54	4.08	3.18	3.56	0.30	1.24	0.33	0.52
Daucus carota	1.83	1.76	3.15	2.13	0.79	2.69	0.51	1.06
Galium odoratum	2.98	2.74	2.51	2.73	0.20	0.61	0.26	0.32
Geum urbanum	6.30	7.79	7.53	7.30	1.13	4.36	0.82	1.56
Heracleum sphondylium	3.25	3.27	3.77	3.42	0.53	2.20	0.62	0.95
Knautia arvensis	12.38	11.95	9.37	11.18	0.72	2.43	0.54	1.03
Oenothera biennis	1.84	1.95	2.56	2.08	0.82	2.82	0.48	1.06
Prunella vulgaris	1.02	1.40	1.61	1.34	0.68	2.51	0.69	1.08
Ranunculus acris	1.82	2.84	3.14	2.51	0.76	3.04	0.61	1.16
mean (Fr)	4.43 a	4.90 a	4.95 a	4.70	0.63 a	2.33 b	0.51 a	0.93

Fr, Friedman test and multiple comparisons with α= 0.05. Letters indicate different groups (Galloway cattle: n = 12; chi^2 = 2.17, df = 2, P=0.338; Haflinger horse: n = 12; chi^2 = 18.17, df = 2, P<0.001).

Role of seed morphology

The correlations between the mean half-life retention times of the seeds and the values of their quantitative seed traits, contrasted between the two herbivore species (Table 5.4). In the case of Galloway cattle, the overall retention times were significantly positively correlated with appendage number and appendage length, while these traits were not correlated with overall retention times on Haflinger horse. Seed size-related traits (especially seed weight) correlated negatively with retention on horse, and positively with retention on cattle.

Table 5.4 *Correlation between quantitative seed traits and mean half-life retention times of seeds on Galloway cattle and on Haflinger horse in three vegetation types (average of two weather conditions) (n=12).*

	Galloway cattle				Haflinger horse			
	all vegetations	forest	tall herbage	meadow	all vegetations	forest	tall herbage	meadow
seed surface texture	0.32 ns	0.25 ns	0.21 ns	0.28 ns	-0.50 ns	-0.32 ns	-0.43 ns	-0.60 *
appendage number	0.58 *	0.46 ns	0.54 ns	0.73 **	0.18 ns	0.22 ns	0.23 ns	0.08 ns
appendage length	0.61 *	0.51 ns	0.57 ns	0.77 **	0.33 ns	0.35 ns	0.37 ns	0.23 ns
seed length	0.76 **	0.69 *	0.73 **	0.78 **	-0.27 ns	-0.38 ns	-0.30 ns	0.04 ns
seed width	0.59 *	0.43 ns	0.52 ns	0.48 ns	-0.53 ns	-0.62 *	-0.56 ns	-0.32 ns
seed thickness	0.53 ns	0.48 ns	0.50 ns	0.29 ns	-0.52 ns	-0.48 ns	-0.45 ns	-0.75 **
seed weight	0.75 **	0.69 *	0.67 *	0.58 *	-0.66 *	-0.76 **	-0.69 *	-0.41 ns

*Values are Spearman rank correlation coefficients (**, P<0.01; *, P< 0.05; ns, not significant).*

Discussion

This study demonstrates that large herbivores such as Haflinger horse and Galloway cattle can be very effective dispersers of plant seeds. On average, about 20% of the experimental seeds of all 12 species remained in the fur of Galloway cattle after 20 hours of observation in natural conditions, and after 3 hours in the fur of Haflinger horse (Fig. 5.1). Although the probability of seeds to become detached from an animal is specific for particular environmental or behavioural features (see also Bullock & Primack 1977), our treatment of the seed depletion curves as smooth curves, which is a conceptual and mathematical convenience, could be justified for most cases, given the high and significant R^2 values (see App. 5.1).

Effect of animal, vegetation, weather and seed morphology

The significant effect of animal species (Table 5.2), which was reflected in the half-life seed retention times (Table 5.3), is undoubtedly related to their fur characteristics (see also Shmida & Ellner 1983). The long, undulated and open fur of Galloway cattle is much more suited for long-distance transport of seeds than the short, straight and closed fur of horse (see also chapters 2 and 4). The retention times on horse are comparable to the observed retention times on short-furred domestic cattle in the studies of Kiviniemi (1996) and Kiviniemi & Eriksson (1999), which indicates that for seed retention, fur type is much more important than animal species (see also chapter 4). However, also other animal-related factors may influence seed retention. Grooming, for instance, is known to accelerate seed depletion, especially of large and irritating seeds (Sorensen 1986, Kiviniemi & Telenius 1998). Since the observation series in this study included walking distances on a lead, as well as a resting period and a free overnight rest, the natural behaviour of the herbivores such as grooming, rubbing, wallowing, lying, running, and interacting with other animals was indirectly included in the experiment.

The half-life seed retention times -ranging from 1.02 h to 12.83 h on Galloway cattle and from 0.19 to 4.35 h on horse- were in general not different between the three vegetation types (Table 5.3). Only on horse, the average seed retention was -surprisingly- higher in tall herbage vegetation. The absence of a general effect of vegetation type on seed retention in the long, undulated fur of Galloway may be explained by the fact that physical contact between animals and vegetation structures might act twofold. On the one hand, branches and stems of plants can sweep away seeds from the fur, while on the other hand such contact can result in the protection of seeds from detachment if the hairs are ‘brushed’ over the seeds by the vegetation (as was sometimes observed). This effect may be more pronounced if long-haired furs are involved. Such contrasting effects, which may often occur in forests and tall herbage vegetations, could neutralize the net effect of vegetation structure on seed retention in long furs. A similar reasoning might explain the absence of a significant weather effect on seed retention in fur. Soft rain may enable seeds to stick better to the wet hairs on the one hand, but may on the other hand render the fur less penetrable for seeds. Possibly, the mucus-secreting seeds of *Prunella vulgaris*, which normally become sticky in rainy weather conditions, may have been less adhesive as a consequence of paint-marking. In other studies, however, the impact of weather (Graae 2002) and vegetation type (Bullock & Primack 1977) on seed retention was more pronounced.

The relation between seed morphology and epizoochorous retention time differed between Galloway cattle and Haflinger horse (Table 5.4). In the long cattle fur, the presence of long and numerous adhesive appendages apparently enhanced seed retention. Surprisingly, also seed weight and size were positively correlated with retention in the Galloway fur. Large and heavy seeds may fall more easily between the locks of the long fur than small and light seeds, and may be as such more protected from detachment. In contrast, small and light seeds were retained better than large and heavy seeds in the short horse fur, which does not allow any seed penetration. Possibly, certain seed traits impact differently on seed retention than on seed attachment. This is suggested by the fact that, on Galloway fur, large seeds attach less easily than small seeds (chapter 4), while once attached, the retention of large seeds seems not to be problematic at al (Table 5.4). The observation of large seeded species in fur of Galloway cattle (chapter 2) confirms that large seeds are able to disperse epizoochorously.

In general, other authors found positive effects of adhesive appendages on seed retention (Shmida & Ellner 1983, Kiviniemi & Telenius 1998, Kiviniemi & Eriksson 1999, Graae 2002), and negative effects of seed size (Sorensen 1986) and seed weight (Kiviniemi & Telenius 1998). Our results, however, suggest that the functionality of adhesive appendages or other seed traits might be related to a certain fur length or fur quality (interaction effect between seed morphology and fur type, see also chapter 4). Interpretation of the results of this study in terms of exact species, however, should be done with caution. The paint-marking might have influenced the adhesive properties of the seeds, and for some species (*Agrimonia eupatoria, Cynoglossum officinale, Galium odoratum)*, the adhesive appendages were shortened or even removed as a consequence of commercial harvesting methods, so they had to be treated as seeds with short or even without appendages (see Table 5.1). It seems therefore appropriate to consider the experimental seeds used in this study as ‘models’ for seeds with similar morphological characteristics (as described in Table 5.1), instead of focusing on the exact species itself.

Seed dispersal distances

The minimal dispersal distance of the experimental seeds 'surviving' the retention experiments was 500 m (the fixed distance included in the walks), but during the overnight rest the animals were free to move, so the real distances of seed movement were probably much larger. Curve fitting of the seed depletion curves (Fig. 5.1, App. 5.1) and subsequent calculation of half-life retention times of all seed species in the different conditions (Table 5.3) allowed a theoretical assessment of the potential half-life dispersal distances. If movement rates of 4 m min^{-1} for grazing large herbivores were assumed (see Kiviniemi 1996), the calculated dispersal distances of at least half of the seeds ranged from 245 to 3080 m for Galloway cattle, and from 47 to 1046 m for Haflinger horse. These results refer of course to seeds positioned on the back and upper flank of the animals. Seeds on more vertically oriented body parts of animals will probably have shorter retention times (see also Bullock & Primack 1977, Graae 2002), while seeds located for instance in the mane of a horse might be transported much further than the calculations in this study suggest. Nevertheless, the order of magnitude of the extrapolated dispersal distances in this study (tens of metres to kilometres) is similar to the results of other seed retention studies on large animals, e.g. sheep and goat (Shmida & Ellner 1983), sheep (Fischer et al. 1996), cattle and fallow deer (Kiviniemi 1996), cattle (Kiviniemi & Eriksson 1999), and dog (Graae 2002).

Ecological significance of seed retention

Assuming potential half-live dispersal distances of seeds in the order of magnitude of tens of metres to a few kilometres by large animals implies that a small proportion of seeds in the tail of the dispersal curve can occasionally be transported over considerably longer distances (see also Graae 2002). In the case of wind dispersal, the least viable seeds (which are lighter) are generally dispersed further (Strykstra et al. 1998b), but this is not likely to be the rule for epizoochory (see Table 5.4). The small portion of seeds in the tail of the dispersal curve might be extremely important in determining plant spread rates. Higgins & Richardson (1999) have even demonstrated that predicted spread rates increased with an order of magnitude if 0.1% of the seeds were dispersed over distances of 1-10 km. The importance of such long-distance dispersal and so-called 'single dispersal events', which can lead to the founding of new populations, has been inferred from studies on Holocene plant migration rates (Clark et al. 1998, Pakeman 2001). An additional advantage of epizoochory is that it provides a reasonable chance of directed dispersal, since animals frequent similar habitats and create suitable germination sites by disturbing the established vegetation (Janzen 1984, Stiles 2000, Wenny 2001).

Implications for nature management

In the present western-European situation of habitat degradation, fragmentation and restriction of natural dispersal processes, it is crucial to restore and enhance the dispersal processes that are left (Bakker et al. 1996, Bonn & Poschlod 1998, Poschlod & Bonn 1998, Bakker & Berendse 1999). Given the considerable potential of large herbivores as 'mobile links' (see Lundberg & Moberg 2003, chapter 2) between isolated habitat patches by means of seed dispersal, measures should be taken and implemented to restore and increase the animals' potential home ranges and their possibilities to migrate across landscapes (Briers 2002, Lundberg & Moberg 2003). In areas where no wild large animals occur, the introduction of large herbivores such as (preferably long-furred) cattle and horses as part of nature management might significantly contribute to the sustainable conservation of many plant species.

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App. 5.1 *Parameters resulting from curve fitting of a negative exponential function on ln-transformed seed depletion data (df = 4). Equation: number of seeds = exp (-b x t).*

	Galloway all vegetations			*Galloway forest*			*Galloway tall herbage vegetation*			*Galloway meadow*		
	Rsq	*F*	*-b*	*Rsq*	*F*	*-b*	*Rsq*	*F*	*-b*	*Rsq*	*F*	*-b*
Agrimonia eupatoria	*0.868*	*105****	*-0.076*	*0.923*	*42**	*-0.094*	*0.917*	*44**	*-0.086*	*0.799*	*16**	*-0.054*
Anemone nemorosa	*0.825*	*75****	*-0.081*	*0.636*	*7 (*)*	*-0.083*	*0.879*	*29**	*-0.064*	*0.937*	*59**	*-0.101*
Centaurea jacea	*0.783*	*58****	*-0.281*	*0.786*	*15**	*-0.286*	*0.691*	*9(*)*	*-0.325*	*0.886*	*31**	*-0.238*
Cynoglossum officinale	*0.913*	*168****	*-0.195*	*0.951*	*77**	*-0.196*	*0.893*	*33**	*-0.17*	*0.902*	*37**	*-0.218*
Daucus carota	*0.767*	*53****	*-0.325*	*0.839*	*21**	*-0.379*	*0.803*	*16**	*-0.394*	*0.771*	*13(*)*	*-0.22*
Galium odoratum	*0.869*	*106****	*-0.254*	*0.676*	*8(*)*	*-0.233*	*0.98*	*195***	*-0.253*	*0.891*	*33**	*-0.276*
Geum urbanum	*0.852*	*92****	*-0.095*	*0.715*	*10(*)*	*-0.11*	*0.784*	*15(*)*	*-0.089*	*0.987*	*295****	*-0.092*
Heracleum sphondylium	*0.926*	*202****	*-0.203*	*0.912*	*42**	*-0.213*	*0.926*	*50***	*-0.212*	*0.945*	*68**	*-0.184*
Knautia arvensis	*0.829*	*78****	*-0.062*	*0.834*	*20**	*-0.056*	*0.699*	*9**	*-0.058*	*0.934*	*56***	*-0.074*
Oenothera biennis	*0.762*	*51****	*-0.333*	*0.784*	*15**	*-0.376*	*0.851*	*23**	*-0.356*	*0.656*	*8(*)*	*-0.271*
Prunella vulgaris	*0.704*	*38****	*-0.518*	*0.681*	*9(*)*	*-0.678*	*0.738*	*11**	*-0.495*	*0.749*	*12**	*-0.432*
Ranunculus acris	*0.697*	*37****	*-0.276*	*0.613*	*6 (*)*	*-0.38*	*0.804*	*16**	*-0.244*	*0.764*	*13(*)*	*-0.221*

	horse all vegetations			*horse forest*			*horse tall herbage vegetation*			*horse meadow*		
	Rsq	*F*	*-b*	*Rsq*	*F*	*-b*	*Rsq*	*F*	*-b*	*Rsq*	*F*	*-b*
Agrimonia eupatoria	*0.904*	*151****	*-1.806*	*0.993*	*609****	*-3.495*	*0.997*	*1524****	*-0.929*	*0.992*	*526****	*-2.364*
Anemone nemorosa	*0.761*	*51****	*-0.635*	*0.966*	*112****	*-0.921*	*0.988*	*323****	*-0.234*	*0.996*	*987****	*-1.3*
Centaurea jacea	*0.772*	*54****	*-0.732*	*0.945*	*69***	*-1.087*	*0.989*	*356****	*-0.293*	*0.99*	*401****	*-1.461*
Cynoglossum officinale	*0.845*	*87****	*-1.34*	*0.993*	*597****	*-2.316*	*0.994*	*692****	*-0.558*	*0.998*	*1609***	*-2.079*
Daucus carota	*0.762*	*51****	*-0.654*	*0.944*	*68***	*-0.879*	*0.99*	*380****	*-0.258*	*0.986*	*283****	*-1.369*
Galium odoratum	*0.932*	*218****	*-2.168*	*0.996*	*956****	*-3.495*	*0.968*	*121****	*-1.128*	*0.996*	*1066****	*-2.668*
Geum urbanum	*0.778*	*56****	*-0.445*	*0.967*	*119****	*-0.616*	*0.987*	*295****	*-0.159*	*0.996*	*891****	*-0.841*
Heracleum sphondylium	*0.815*	*71****	*-0.73*	*0.982*	*215****	*-1.307*	*0.98*	*201****	*-0.315*	*0.997*	*1372****	*-1.112*
Knautia arvensis	*0.794*	*62****	*-0.675*	*0.9517*	*79****	*-0.969*	*0.97*	*164****	*-0.285*	*0.997*	*1386****	*-1.29*
Oenothera biennis	*0.749*	*48****	*-0.653*	*0.9531*	*81****	*-0.846*	*0.978*	*182****	*-0.246*	*0.99*	*414****	*-1.459*
Prunella vulgaris	*0.754*	*49****	*-0.64*	*0.93*	*53****	*-1.026*	*0.929*	*53****	*-0.276*	*0.93*	*53****	*-1*
Ranunculus acris	*0.772*	*54****	*-0.598*	*0.961*	*98****	*-0.907*	*0.993*	*558****	*-0.228*	*0.994*	*660****	*-1.139*

**** P<=0.001; ** P<=0.01; * P<= 0.05; (*) P<= 0.085*

Chapter 6

Epizoochory by large herbivores: merging data with models

Abstract

The dispersal of plant seeds in animal fur (epizoochory) is an important but complex long-distance dispersal mechanism. We developed a spatially explicit simulation model of epizoochorous seed dispersal, which was parameterized based on empirical studies of the movement and behaviour of donkeys, and the distribution, seed production, seed accessibility, seed adhesion, and seed retention on donkey fur of selected plant species in a coastal dune nature reserve in Flanders, Belgium. To test the model, we compared predicted and observed seed numbers of the 14 plant species on donkey fur. Modelled seed shadows indicate that for most species about half of all seeds dispersed by donkeys should travel a net distance of >100 m, and about 1% should travel >800 m within this more or less isodiametric 80 ha nature reserve. Seeds with longer retention times are expected to travel further than those with short retention times. Variations among species in the observed seed numbers found on donkey fur were surprisingly well predicted by the model (R^2=0.56, P=0.002) given the relatively crude estimates of seed production and accessibility to donkeys. Our model confirms the important role of epizoochory in effecting long-distance seed dispersal, and provides a modelling framework for integrating the multiple components of the dispersal process.

Keywords

Donkey, epizoochory, fragmented dune landscape, fur, grazing, herbivore movement, nature management, plant distribution, seed dispersal, spatially explicit simulation model

Introduction

Seed dispersal is a key factor in plant spatial dynamics, influencing the distribution of populations and communities, both at local and regional scales (e.g. Cain et al. 2000, Bullock et al. 2002, Levin et al. 2003, Ozinga et al. 2004). Range shifts of plants are determined largely by long-distance seed dispersal, as demonstrated in the context of Holocene plant migrations (Clark 1998, Clark et al. 1998, Higgins & Richardson 1999, Cain et al. 1998, Pakeman 2001), and in the context of actual and future plant migrations in response to land use changes (e.g. Matlack 1994, Poschlod & Bonn 1998, Vellend et al. 2003, Takahashi & Kamitani 2004) and climate change (Walther et al. 2002, Watkinson & Gill 2002, Walther 2004).

Seed availability is a major factor influencing plant distributions in a variety of ecosystems (e.g. Zobel et al. 2000, Turnbull et al. 2000, Dalling et al. 2002, Foster & Tilman 2003, Verheyen et al. 2003). Also the functional connectivity between habitat patches, a crucial aspect of plant metapopulation dynamics, depends on long-distance dispersal (Eriksson 1996, Cain et al. 2000). The relatively poor dispersal abilities of many native plants (e.g. forest herbs, Bossuyt et al. 1999, Verheyen et al. 2003) as compared to some invasive species (e.g. Hodkinson & Thompson 1997, Malo & Suárez 1997), make clear that global change will drastically reshuffle species assemblages. Considering the present degree of habitat deterioration and fragmentation throughout the world, the long-term survival of many plant species and communities hence depends on successful long-distance dispersal. This prospect highlights the need for predictive models of plant dispersal, which generate realistic behaviour, and which are validated with field observations (Levin et al. 2003). Several authors (e.g. Higgins & Richardson 1999, Nathan & Muller-Landau 2000) have emphasized the critical importance of long-distance dispersal in addressing the discrepancy between observed and predicted spread rates of plants (Clark et al. 1998). Also models of metapopulation dynamics rely on an adequate assessment of the long-distance dispersal component. The prediction of seed shadows could be very useful in ecological restoration projects, where the establishment of new plants often depends on long-distance dispersal. Models of long-distance dispersal should of course be validated with field data on long-distance dispersal (Higgins & Richardson 1999). ‘Long-distance’ is obviously a relative term, though seed dispersal >100 m is generally considered long-distance dispersal (Cain et al. 2000).

Among the major long-distance dispersal mechanisms, the transport of seeds in animal fur (epizoochory) receives increasing attention (Fischer et al. 1996, Kiviniemi 1996, Stender et al. 1997, Kiviniemi & Telenius 1998, Mrotzek et al. 1999, Heinken 2000, Graae 2002, Heinken & Raudnitschka 2002). However, compared to e.g. wind dispersal (Greene & Johnson 1989, Bullock & Clarke 2000, Tackenberg et al. 2003), attempts to model zoochorous dispersal are relatively scarce, likely because of the inherent difficulty of tracing zoochorous long-distance dispersal, and the associated problem of incorporating this component, which is influenced by complex animal behaviour, into a model (Nathan & Muller-Landau 2000). Vellend et al. (2003) and Higgins et al. (2003a) have recently used data on animal movement patterns and seed retention to model endozoochory by white-tailed deer in eastern North America, and epizoochory by lynx in southwestern Spain, respectively. However, these models did not take account of the influence of habitat preferences on animal movement patterns, nor did they allow for predictions of variation among plant species in the number of seeds dispersed. Considerable challenges in constructing and parameterizing models of zoochorous seed dispersal therefore remain.

Here, we present a spatially explicit simulation model of epizoochorous seed dispersal, parameterized and tested based on empirical data concerning most key features of the dispersal process. The model simulates the movement of donkeys in a fenced coastal dune nature reserve, and the simultaneous uptake and epizoochorous dispersal of seeds of 14 local plant species. Our two principal objectives were (1) to estimate seed shadows for plant species with different seed retention characteristics on animal fur, and (2) to test the model by attempting to predict the number of seeds of each species found on donkey fur in the field.

Material and Methods

Study site and vegetation

The study site was the 80 ha coastal dune nature reserve ‘Houtsaegerduinen’, in western Flanders, Belgium (51° 05’ N, 2° 35’ E) (Fig. 6.1). For nature management purposes, six donkeys were released in the reserve in 1997. The herd comprised 15 animals in 2000, the period of data collection. The variation in abiotic conditions and the historical land use in this coastal dune ecosystem have led to a relatively high plant species richness and a range of different plant communities. Flowering and fruiting of the plant species in the study site is concentrated from April to October. Vegetation surveys (using Tansley scaled ordinal abundance estimates in combination with percentage cover estimates in $1m^2$ quadrats, collected on several occasions between 1990-2000), were used to divide the study area into four main vegetation types: shrub, forest, grassland including moss dunes, tall herbage vegetation (see Fig. 6.1).

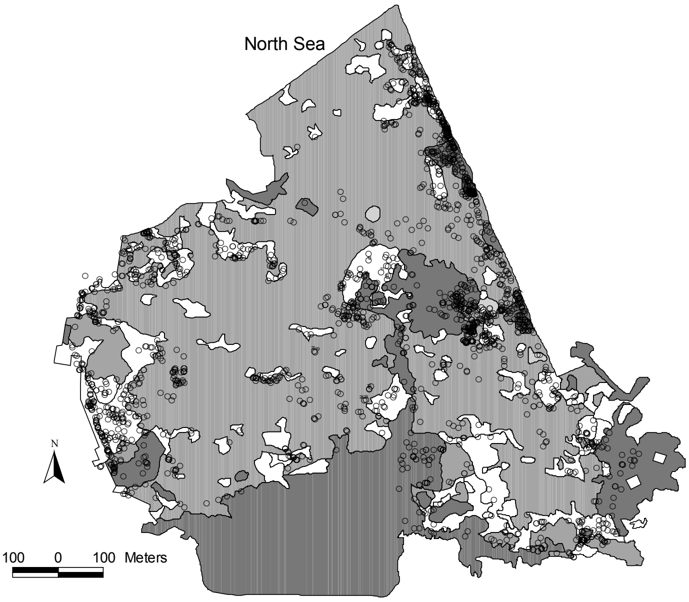

Fig. 6.1 *Study site, the 80 ha Flemish nature reserve 'Houtsaegerduinen' along the North Sea coast. Four main vegetation types are indicated with different colours (grassland and moss dune, white; tall herbage vegetation, light grey; shrub, intermediate grey; forest, dark grey). Black circles represent locations of donkeys, recorded with 15 min intervals.*

The most abundant vegetation type was shrub, dominated by *Hippophae rhamnoides* and *Ligustrum vulgare,* and to a lesser extent *Prunus spinosa* and *Crataegus monogyna*. Forest was the second most abundant vegetation type, dominated by planted *Alnus glutinosa* and *Populus x canadensis* trees. The third most abundant was the 'open' vegetation type, consisting of grassland and moss dunes. The grassland generally occurs as scattered patches and represents species poor remnants of dune grassland, characterized by low-growing *Rosa pimpinellifolia* and several grasses *(Avenula pubescens*, *Arrhenatherum elatius, Poa pratensis)*. Typical but rare species in dune grasslands are *Thymus pulegioides, Helianthemum nummularium* and *Silene nutans*. The moss dunes were characterized by mosses and lichens *(Tortula ruralis* ssp. *ruraliformis, Hypnum cupressiforme* var. *lacunosum, Cladonia* spp.), in combination with *Carex arenaria* and *Koeleria albescens*. Finally, the least abundant vegetation type was 'tall herbage', typically established after scrub degradation, and existing of rather species poor *Calamagrostis epigejos* - *Arrhenatherum elatius* dominated patches with local stands of *Eupatorium cannabinum* and *Epilobium angustifolium*.

Model

Estimating epizoochorous seed shadows requires information on animal movement patterns and seed retention times. The movement pattern (i.e., the probability that an animal will be at different distances from the point of seed adhesion at different times following adhesion) depends, in turn, on the rate of animal movement and its behaviour with respect to different habitat types it may travel through. Predicting the average number of seeds adhering to an animal over time requires additional knowledge of the spatial distribution, seed production, seed accessibility, and seed adhesion to animal fur of the different plant species. In our model we incorporated data on these components derived from our own empirical studies, from the literature, and from inferences based on plant morphology. The model (see App. 6.1) was implemented in Matlab 6.5 (Matlab 2002), and the first step was to convert a vegetation map of the study area into a grid of 10 x 10 m cells, with each cell assigned to one of the four main vegetation types (shrub, forest, grassland/moss dune, tall herbage).

Animal movement

Movement rate and habitat preference of donkeys in the study site were derived from observational data (see Fig. 6.1), recorded during 32 observation sessions, conducted between 3 May 2000 and 25 June 2001, spread over the four seasons and more or less evenly distributed between morning (6-12 h), afternoon (12-18 h) and evening (18-24 h) (see Lamoot et al. in press). Each session consisted of a 5 h 45 min visual observation period on one randomly chosen focal animal of the herd, whose exact position was located on a map every 15 min (total locations = 768). The Euclidean distance between each consecutive donkey location was calculated, resulting in a vector of 'distances covered in 15 min'. To obtain a donkey habitat preference factor for each of the four main vegetation types in the study site, the proportion of observed donkey locations in each vegetation type was divided by the proportional area of that vegetation type. The habitat preference factors were then rescaled to numbers between 0 and 1, by dividing them by the highest obtained habitat preference factor. These rescaled values were named ‘permeability coefficients’ (reflecting the relative permeability of the four vegetation types for the donkeys, see Table 6.1) and were assigned to all 10 x 10 m cells according to their vegetation type.

Table 6.1 *The four main vegetation types in the study site, their proportional area (total area = 80 ha) and the observed and simulated proportional visits of the donkeys (total visits = 768). The ratio of these figures gives the habitat preference factor of the donkeys, or after rescaling to a maximum of 1, the permeability coefficient of each vegetation type.*

vegetation type	proportion of area	proportion of visits: observed (simulated)	habitat preference factor	permeability coefficient
shrub	0.58	0.38 (0.40)	0.66	0.262
forest	0.25	0.25 (0.25)	0.99	0.394
grassland + moss dunes	0.13	0.33 (0.31)	2.51	1.000
tall herbage	0.04	0.03 (0.04)	0.97	0.385

The movement of donkeys in the reserve was simulated by a restricted random walk, in which the distance covered in one 15 min step was randomly selected from the observed vector of ‘distances covered in 15 min’. The direction of each movement step was chosen randomly, but a movement step was only accepted with probability equal to the vegetation-specific permeability coefficient (cells outside the fence were accepted with probability zero). A new random direction was drawn until the movement step was accepted. Initial results of the movement model (1000 simulations of 5 h 45 min) were checked for consistency with empirical data in two ways. First, we compared the predicted and observed proportions of time spent in each habitat type. Second, we compared predicted and observed probabilities of finding a donkey at different distances from its starting point during 15 min time intervals. Both comparisons revealed a close correspondence between model predictions and empirical observations (see Table 6.1 and Fig. 6.2).

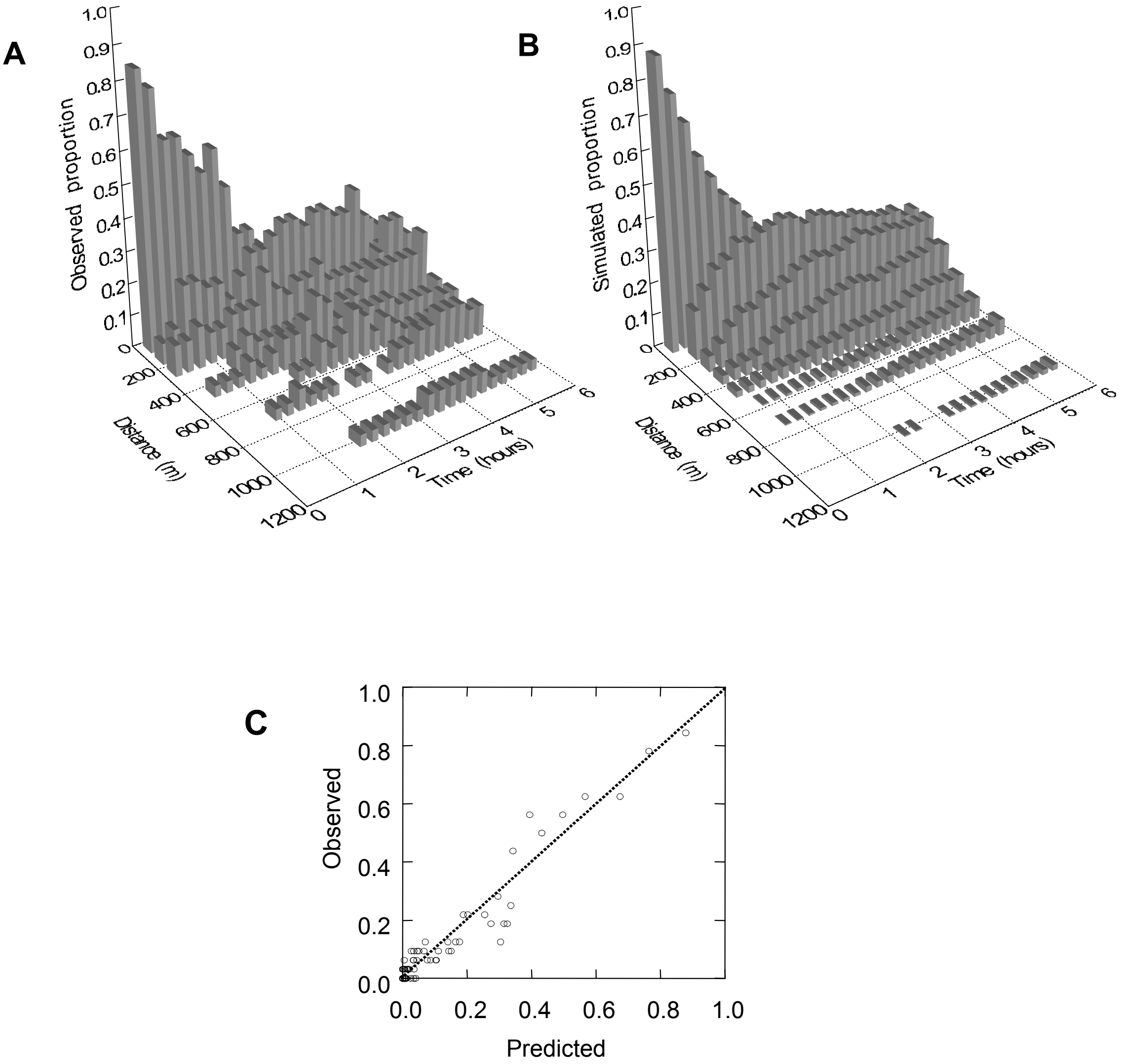

Fig. 6.2 *Observed (A) and simulated (B) proportion of positions when a donkey is at different distances from its starting point during 15 min time intervals. (C) Relationship between observed and simulated data from A and B. Observed donkey movement patterns were characterized by tabulating the proportion of positions across the 32 observation periods (5 h 45 min) during which the focal animal was within eight distance classes from its starting point (0-100, 1-200, 200-300, 300-400, 400-500, 500-600, 600-800, 800-1200m) in 15 min intervals (Fig. 6.2A). An identical procedure was applied to the results of 1000 simulations of 5 h 45 min each (Fig. 6.2B). Observed data were tightly correlated with predicted (R^2=0.82, P<0.001; Fig. 6.2C).*

Therefore, we used the above-described restricted random walk to model donkey movement for longer time periods. For use in estimating seed shadows, 1000 simulations of 20 h each (20 h corresponds to the maximum seed retention time) were conducted to build the **MOV** matrix, in which each element represents the probability that a donkey has travelled a given net distance (in 100 m intervals up to 1200 m) at different times since the beginning of the simulation (in 15 min intervals, see also Vellend et al. 2003). For estimating seed numbers adhering to donkeys, we conducted 5 simulations of 1000 time steps (10.4 days) for each of 14 plant species.

Table 6.2 *Abundance (abun) and frequency (freq) of the 14 model plant species in the four main vegetation types of the study site. The frequency is expressed as the proportion of 10x10 m cells occupied, while the abundance represents the number of plants per occupied 10x10 m cell.*

		grass & moss dunes		forest		shrub		tall herbage	
species	code	freq	abun	freq	abun	freq	abun	freq	abun
Galium aparine	gaap	0.055	3	0.1	5	0.3	5	0.3	5
Cynoglossum officinale	cyof	0.015	1	0.1	5	0.1	5	0.2	5
Arctium minus	armi	0	0	0	0	0.05	1	0.05	1
Anthriscus caucalis	anca	0	0	0	0	0.1	10	0.1	10
Oenothera biennis	oebi	0.15	8	0	0	0	0	0.05	5
Urtica dioica	urdi	0.05	10	0.4	500	0.3	30	0.3	50
Arrhenatherum elatius	arel	0.45	500	0.1	50	0.2	50	0.9	1000
Senecio jacobaea	seja	0.2	30	0	0	0	0	0.05	2
Myosotis arvensis	myar	0.26	100	0	0	0	0	0	0
Poa trivialis	potr	0.15	40	0.1	5	0.1	5	0.2	100
Lythrum salicaria	lysa	0	0	0	0	0	0	0.02	5
Cerastium semidecandrum	cese	0.48	80	0	0	0	0	0	0
Holcus lanatus	hola	0.1	50	0.1	1	0.1	5	0.3	100
Festuca rubra	feru	0.15	40	0	0	0	0	0	0

Plant species, distribution and seed production

Among the 335 plant species present in the study site, the 14 most abundant species germinating from epizoochorous seed loads of the donkeys (see chapter 3) were selected for our simulations. Several seed types were represented among the 14 species (e.g. hooked, awned, with a pappus, small and unspecialized, see Table 6.4). The distribution and abundance of each species in each of the four main vegetation types in the study area was recorded in the field and included in the model as the proportion of 10 x 10 m cells occupied by the species, and the number of plants per occupied cell (Table 6.2). Approximate seed numbers per plant were derived from the literature (Table 6.3). Simulations were conducted for one species at a time, and the species' abundance data were provided for each vegetation type separately (Table 6.2).

Table 6.3. *Approximate seed number per plant and associated reference; plant height and degree of exposure of the seeds of each model species, reflected in the accessibility factor of the seeds (which is multiplied by the number of seeds to obtain the number of accessible seeds in a given 10 x 10 m area); average number of seeds observed in fur of donkeys in study site.*

species	seeds per plant	reference of seeds per plant	plant height (cm) (Grime et al. 1988)	degree of seed exposure	accessi-bility factor	accessible seed number	observed seeds in fur
Galium aparine	300	Poschlod et al. 2003	60-99	exposed	1	300	52.2
Cynoglossum officinale	150	Melser and Klinkhamer 2001	30-59	exposed	1	150	29
Arctium minus	10000	Poschlod et al. 2003	100-300	exposed	1	10000	19.6
Anthriscus caucalis	150	/	30-59	exposed	1	150	18
Oenothera biennis	15000	Mihulka et al. 2003	60-99	enclosed	0.1	1500	10
Urtica dioica	500	Poschlod et al. 2003	60-99	covered	0.1	50	9.9
Arrhenatherum elatius	40	Grime et al. 1988	100-300	panicle	0.1	4	2.4
Senecio jacobaea	10000	Grime et al. 1988	60-99	enclosed	0.1	1000	2
Myosotis arvensis	500	Poschlod et al. 2003	10-29	enclosed	0.01	5	1.9
Poa trivialis	500	Poschlod et al. 2003	30-59	panicle	0.1	50	1.3
Lythrum salicaria	15000	Ture et al. 2004	60-99	enclosed	0.1	1500	0.9
Cerastium semidecandrum	250	Poschlod et al. 2003	10-29	enclosed	0.01	2.5	0.5
Holcus lanatus	150	Poschlod et al. 2003	30-59	panicle	0.1	15	0.2
Festuca rubra	100	Grime et al. 1988	10-29	panicle	0.1	10	0.2

Seed dispersal

If the animal entered a cell that was occupied by the focal plant species, it potentially came into contact with all accessible seeds of all plants present in that cell. Since the degree of ‘accessibility’ of a plant’s seeds for epizoochory depends on the degree of seed exposure and the height of the plant (cf. Fischer et al. 1996), the number of accessible seeds per plant was calculated by multiplying seed number per plant by a seed ‘accessibility factor’. This factor (logarithmically scaled, and arbitrarily ranging between 0.01 and 1) was high if the seeds were clearly exposed and/or positioned on a tall stem; the factor was low if the seeds were not exposed and/or located on a very short stem (Table 6.3). The resulting ‘accessible seed number’ represented the fraction of the total seed number potentially available for epizoochorous dispersal. Because its values are arbitrary, our accessibility factors might allow only qualitative discrimination among species with different morphologies (relative variation in numbers of seeds per species on a donkey), rather than a quantitative prediction of actual numbers of seeds per species. It is important to emphasize that the accessibility factors were determined *a priori* based on knowledge of plant traits.

Seed uptake and seed loss were modelled using plant-specific parameters obtained from experimental data on seed adhesion and seed retention on horse fur. This extrapolation can be justified, since the behaviour of donkeys and horses is quite similar (Cosyns et al. 2001) and since donkey fur is at least as suited for seed dispersal than horse fur, given the fur characteristics of both animals (see also chapter 4). If no experimental data for the 14 plant species were available, the selected parameters were taken from species with morphologically or functionally similar seeds (Table 6.4). The main criteria for the selection of representative seeds were the presence of adhesive appendages, seed shape and seed size. Although for some seed types no perfect representatives were available, this procedure allowed us to include considerably more species in the analysis. Seed uptake was modelled using a seed adhesivity score (Table 6.4), which reflects the attachment capacity of seeds to fur. These scores were experimentally quantified as the proportion of seeds applied to horse fur that remained attached (see chapter 4). Nine different adhesivity scores were used to represent the 14 focal plant species in this study (Table 6.4). From the model species represented by species with morphologically or functionally similar seeds, the most adhesive ones (with hooks) were represented by *Erodium cicutarium*; the medium sized grass seeds without seed appendage by *Festuca pratensis*, and the tiny seeds of *Cerastium* *semidecandrum* by *Myosoton aquaticum*. The subsequent loss of attached seeds from the fur was modelled using experimental data on the retention time of seeds on a moving horse in field conditions over 20 h periods (see chapter 5). To represent the 14 focal plant species in this study, retention parameters from five different plant species were used (Table 6.4). The most adhesive study species (with hooks) were represented by the hooked-seeded *Geum urbanum* (as was *Senecio jacobaea*, since pappus hares are generally quite adhesive, see chapter 2 and 4); the intermediately sized grass seeds without seed appendage by *Centaurea jacea* (and with seed appendage by *Anemone nemorosa)*; the small and irregularly shaped ones by *Oenothera biennis*; and the tiny ones by *Prunella vulgaris*. The species-specific seed retention parameter was used for all four vegetation types, since it can be assumed that seed retention times do not considerably differ between vegetation types (see chapter 5). The proportion of seeds remaining attached after adhesion (which occurs at time $t = 0$) was modelled as $\exp(b \times t)$, with R^2 ranging from 0.75-0.78 ($P<0.001$) across species. Although some seeds remain attached to horse fur after 20 h (mean across species = 1.9%; chapter 5), the distribution of distances travelled changes very little after 20 h (as a consequence of the fenced study site) so that modelling longer time periods makes no appreciable difference.

Seed shadows and seed numbers

The seed retention curves for each species were used to generate a retention-time vector $\mathbf{RT_S}$ (S refers to the species), in which each cell represents the proportion of seeds dropped in 15 min time intervals from 0-20 h. The species-specific seed shadows were then calculated as $\mathbf{MOV} \times \mathbf{RT_S}$ (see also Vellend et al. 2003).

Independent data on observed seed numbers per plant species on donkeys (from chapter 3) were used to validate model predictions. The seed numbers (shown in Table 6.3) were obtained by inspection of the seeds brushed from the fur of individual donkeys in the study site, at several occasions during the vegetation season of 2000 (see chapter 3). The observed seed numbers were calculated as the mean across samples during the period when the species was present in the fur samples. Predicted seed numbers from the model were calculated as the mean across time during the five 10.4-day simulations. Linear regression on log-transformed values was used to compare predicted and observed seed numbers (SPSS 2001).

Although empirical data were used to parameterize several components of the model, observed seed numbers were entirely independent from predicted numbers.

Table 6.4 *Seed description of model plant species and epizoochory parameters: seed adhesivity score (AS) and seed retention parameter (b), as measured for the model species or available morphologically similar species. The adhesivity score represents the proportion of seeds adhering to horse fur immediately after application (see chapter 4). The b parameter refers to the exponential curve 'Proportion of seeds remaining attached = exp(b*t)', fitted on experimental seed retention data (see horse data from chapter 5).*

model plant species	seed description	AS	source AS	b	source b
Galium aparine	medium, hooked	0.464	*Erodium cicutarium*	-0.445	*Geum urbanum*
Cynoglossum officinale	large, hooked	0.464	*Erodium cicutarium*	-0.445	*Geum urbanum*
Arctium minus	large, hooked	0.464	*Erodium cicutarium*	-0.445	*Geum urbanum*
Anthriscus caucalis	medium, hooked	0.464	*Erodium cicutarium*	-0.445	*Geum urbanum*
Oenothera biennis	small, irregular	0.028	*Oenothera biennis*	-0.652	*Oenothera biennis*
Urtica dioica	small, irregular	0.176	*Urtica dioica*	-0.652	*Oenothera biennis*
Arrhenatherum elatius	medium, awned	0.268	*Arrhenatherum elatius*	-0.635	*Anemone nemorosa*
Senecio jacobaea	medium, pappus	0.16	*Senecio jacobaea*	-0.445	*Geum urbanum*
Myosotis arvensis	tiny, smooth	0.16	*Myosotis scorpioides*	-0.64	*Prunella vulgaris*
Poa trivialis	medium, smooth	0.04	*Festuca pratensis*	-0.732	*Centaurea jacea*
Lythrum salicaria	tiny, smooth	0.364	*Lythrum salicaria*	-0.64	*Prunella vulgaris*
Cerastium semidecandrum	tiny, smooth	0.03	*Myosoton aquaticum*	-0.64	*Prunella vulgaris*
Holcus lanatus	medium, smooth	0.04	*Festuca pratensis*	-0.732	*Centaurea jacea*
Festuca rubra	medium, smooth	0.04	*Festuca pratensis*	-0.732	*Centaurea jacea*

Results

Since seed retention data for five different species studied in chapter 5 were used to represent the range of seed characteristics among the 14 species used in this study (see Table 6.4 and 'Material and Methods' section), five different seed shadow profiles were obtained (Fig. 6.3). All showed approximately exponential decreases in the proportion of seeds dispersed with distance, with mean net seed dispersal distances ranging from 200 to 225 m (Fig. 6.3) (the dispersal distances were restricted by the limited movement possibilities of the donkeys in the fenced nature reserve). Still, seeds with longer retention times (e.g. Fig. 6.3A) should be dispersed further than seeds with shorter retention times (e.g. Fig. 6.3E). For most species about half of all seeds dispersed by the donkeys will travel a net distance of >100 m, and about 1% will travel >800 m within this nature reserve, where the maximum distance between any two points is only 1.4 km.

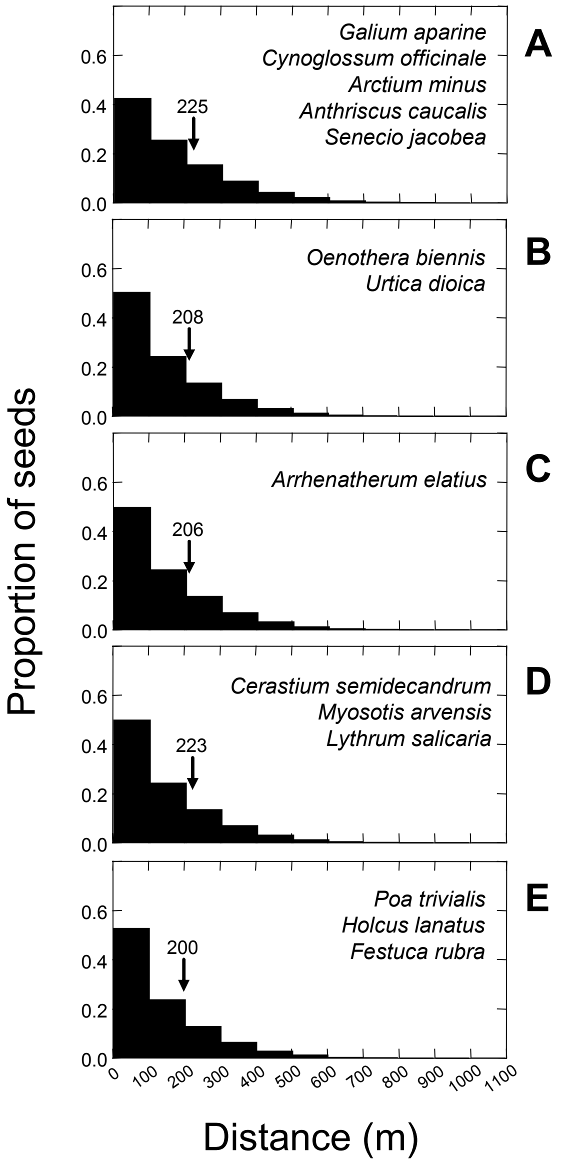

Fig. 6.3 *Modelled seed shadows generated by epizoochory: distribution of net realized dispersal distances of the seeds in donkey fur in the 80 ha study site. Five different seed shadow profiles are modelled because seed retention characteristics for five different species (cf. Table 6.4) were used to represent the 14 species used in this study. Arrows indicate the mean net dispersal distance.*

The number of seeds of each of the 14 plant species in donkey fur was systematically (with approximately one order of magnitude) overestimated by the model (Fig. 6.4). However, given the relatively crude estimates of seed availability, the predicted seed number was a strong and significant predictor of variation in the observed data (F=15.2, R^2=0.56, P=0.002, Fig. 6.4A). The ability of the model to predict variation among species in observed seed number depended on inclusion of the accessibility factor, as revealed by a lack of significant relationship between observed and predicted values when the accessibility factor was excluded (i.e., =1 for all species, F=1.1, R^2=0.08, P=0.315, Fig. 6.4B).

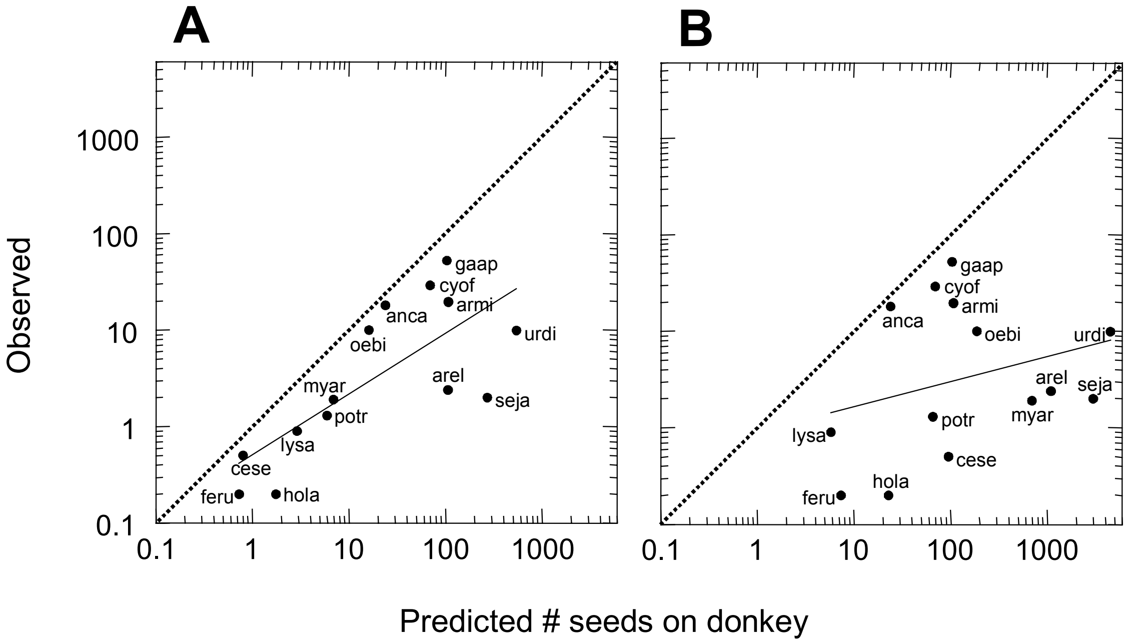

Fig 6.4 *Observed vs. predicted seed numbers in donkey fur of 14 plant species (represented by their abbreviations, see Table 6.2 for full names)* ***A****. using the seed accessibility factor (F=15.2,* R^2*=0.56,* P=0.002*).* ***B****. without the seed accessibility factor (F=1.1,* R^2*=0.08,* P=0.315*).*

Discussion

The simulation model presented in this study (see App. 6.1) allows prediction of the number of plant seeds in animal fur, as well as animal-generated seed shadows for plant species with different seed characteristics. The fact that the model is parameterized with extensive empirical data on most key aspects of the dispersal process, and validated with independent field observations, distinguishes it from other recent models presented in the context of long-distance seed dispersal (e.g. Vellend et al. 2003, Higgins et al. 2003a). Here, the model was applied on donkeys, which are expected to be reasonably representative for other large herbivores (see chapter 2).

Epizoochorous seed shadows

Seeds are likely to be dispersed via epizoochory by donkeys throughout the entire study site, with half of all donkey-dispersed seeds moving further than 100 m from their point of attachment (Fig. 1). At least 29 species in the study site are prone to epizoochorous dispersal (chapter 3), with well over 100 seeds expected to be on a donkey at any one time. The panels in Fig. 1 represent a realistic range of seed shadows that may be generated by donkeys in the study site, with highly adhesive seeds (e.g. *Galium*, *Arctium*, Fig. 1A) showing longer dispersal distances than less adhesive seeds (e.g. *Poa*, *Holcus*, Fig. 1D). The difference in mean modelled realized dispersal distance between the more and the less adhesive seeds is limited by the size of the studied nature reserve, where the Euclidean distance covered by the donkeys (between the place of seed attachment and seed detachment) does not always increases with time, since the donkeys often move in circles. Nevertheless, the modelled seed shadows are larger for the seeds with adhesive adaptations. In combination with higher dispersed numbers of the more adhesive seeds (Fig. 6.4), this may well represent a significant ecological difference.

Given that our study site was a fenced nature reserve, the dispersal distances estimated here are likely to be lower than for wild animals with less constrained movements. Nonetheless, our modelling approach should be generally applicable to systems in which epizoochorous seed dispersal is important, with appropriate adjustments based on local details of animal and plant characteristics. This kind of model can provide a critical building block for modelling plant migration in the face of future landscape changes.

Seed numbers in animal fur

Seed numbers observed on animals are of interest both for determining the importance of epizoochory for different plant species and for providing an independent source of data for testing of the model. Seed numbers in donkey fur were overestimated by the model, typically by one order of magnitude (Fig. 6.4). As the overestimation is relatively similar for all species, the use of a correction factor of 10 should allow quantitative estimations of seed numbers in fur. The overprediction was almost certainly a consequence of our relatively crude determinations of seed number per plant and seed accessibility. Reliable data on seed production per plant are available for only very few plant species, and seed production can vary enormously between contrasting habitats (Nathan & Muller-Landau 2000) and even between years. There has been relatively little study at all of relative accessibility of seeds to seed-dispersing animals. What was surprising was the success with which our model was able to predict variation among species in seed number on donkeys despite these shortcomings. The importance of seed accessibility was clearly indicated by the relatively poor correspondence between model predictions and data in its absence (compare Figs. 6.4 A and B), pointing to an important focus of future studies. Important considerations in assessing accessible seed numbers should take into account animal behaviour with respect to particular plant species, and determination of actual seed numbers in the field rather than ‘typical’ seed numbers derived from compilations. For instance, reproduction of some species (e.g. *Arrhenatherum elatius*) may largely be vegetative rather than sexually due to overgrazing.

Simulating animal movement

Modelling animal movement has been the subject of considerable research. Animal species may perceive the same landscape differently, and are likely to be influenced by different plant characteristics, such as plant species, shape and distribution (Etzenhouser et al. 1998). For simplicity, animal movement is often described by a correlated random walk, depending on three variables: number of steps, step size, and distribution of random turning angles (Byers 2001). The simplest form is an uncorrelated random walk, with a uniform random distribution of turns with a maximum angle of 180° right or left. In this paper, realistic animal behaviour was achieved by combining the mathematically convenient uncorrelated random walk with a donkey movement rate distribution and a simple movement rule based on observational data of habitat preference by the donkeys (Fig. 6.2). The fact that the nature reserve was fenced simplified movement rules relative to animals in unconstrained conditions (free-ranging equids can have home ranges of several hundred hectares, King 2002). However, most large herbivores move predominantly within well-defined home ranges (Worton 1987), suggesting that our model may not be unrealistic even in more complex systems. Higgins et al. (2003), for instance, obtained mean simulated epizoochorous dispersal distances of 572 m for burrs of *Xanthium strumarium* by free-ranging Iberian lynx in a Spanish national park.

Multiple modes of dispersal

The present study focused on the simulation of epizoochorous seed dispersal, although seeds of most plants are dispersed by multiple vectors (e.g. Nathan & Muller-Landau 2000, Higgins et al. 2003b). Rabbits, the most prevalent wild mammals in the study site, probably contribute to epizoochory on a smaller scale, since they have small home ranges (approximately 4 ha, Bond et al. 2001). On the other hand, since the rabbits can reach all microsites in the thorny shrub patches and are not constrained by the fences, they may be important seed-dispersal links with the landscape surrounding the nature reserve. Herbivores such as donkeys and rabbits disperse seeds not only by epizoochory, but also by endozoochory (see e.g. Janzen 1984, Welch 1985, Gardener et al. 1993, Malo & Suárez 1995a, Pakeman 1998, Heinken et al. 2002, chapter 3). In contrast to epizoochory, endozoochory is characterized by an extended lag prior to defecation (approximately 60 h for donkeys, Cosyns et al. in press b), a high loss of seeds through chewing or digestive processes (Cosyns et al. in press b), and seed retention times which are restricted by gut passage time. For the forest herb *Trillium grandiflorum,* which is dispersed endozoochorously by white-tailed deer in eastern North America, average seed shadows of several hundreds of meters have been modelled (Vellend et al. 2003). Another important dispersal vector in dune ecosystems is probably wind. Anemochory is promoted by thermal updrafts, and is more effective for species with a low falling velocity and a high seed release height (Tackenberg et al. 2003). However, seed dispersal distances generated by anemochory are for most plant species restricted to a few metres from the parent plant (Bullock & Clarke 2000, Jongejans & Telenius 2001). Although wind is generally more omnipresent than animal vectors, it may be restricted mainly to the open habitats of the coastal dunes (grasslands and moss dunes).

Implications for nature conservation and management

The present model allows the assessment of the impact of large herbivores on seed dispersal. For ecosystem conservation and restoration purposes, the model can be an instrument to evaluate current dispersal potential of plants, and to guide decisions concerning the introduction of large herbivores. Dispersal vectors provide a critical ecosystem function in a conservation context by linking fragmented patches of natural habitat (see Pykälä 1999, Lundberg & Moberg 2003, Wenny 2003). In our study site, an 80 ha coastal dune landscape, the donkeys clearly disperse seeds among all habitat types throughout the reserve (see also chapter 2). An overlay of the vegetation map with the seed dispersion pattern (combined seed shadows of individual plants) could provide information on the degree of directed dispersal (see Wenny 2001), though this would require much more detailed information on animal movement and behaviour. More generally, our modelling procedure is widely applicable to systems in which quantitative information about epizoochorous seed dispersal is needed.

Acknowledgements

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App. 6.1 *Matlab program used to model epizoochory (parameters of Oenothera biennis in bold).*

```
% This program describes (1) the movement of a large herbivore in a heterogeneous landscape
% and (2) the epizoochorous seed transport of a focal plant species by the animal.
% Sections starting with *** indicate input

clear; % everything should be cleared at the beginning

% *** input of biotope matrix with permeability classes for the 4 prevailing habitat types
% (permeability derived from behavioural studies):
% border + outside fence + enclosures: 0
% grassland and moss dune: 1.00
% forest: 0.394
% tall herbage: 0.386
% shrub: 0.263
% cell size of biotope matrix is 10 m x 10 m

biotope = load('c:\biotopematrix.txt'); % load biotope matrix with permeability classes;
biotopeR = size(biotope,1);
biotopeC = size(biotope,2);

% *** input of distances travelled by animal per time unit (15 min)
% distances are rescaled to cell size (10 m; 1 = 10 m)

animalmovement = load('c:\animalmovement.txt');
% load vector of distances in 10s of m moved in 15’;
animalmovementR = size(animalmovement,1);

% *** Introduction of focal plant species in study area. The focal plant species is distributed
% randomly in a certain biotope, with a defined frequency (proportion of cells occupied),
% and a defined local abundance (number of individuals per occupied cell),
% and a defined seed production;

% input of the frequency and abundance values in the 4 biotopes for the focal plant species;
freqgrass = 0.15; % proportion of cells occupied by the species in grassland-biotope
freqforest = 0.0; % proportion of cells occupied by the species in forest-biotope
freqshrub = 0.0; % proportion of cells occupied by the species in shrub-biotope
freqtallherb = 0.05; % proportion of cells occupied by the species in tall herbage-biotope
abungrass = 8; % number of plants per cell in grassland
abunforest = 0; % number of plants per cell in forest
abunshrub = 0; % number of plants per cell in shrub
abuntallherb =5; % number of plants per cell in tall herbage
seedprod = 1500; % number of seeds per plant * accessibility factor (15000*0.1)

% assigning plant species presence and seed numbers to cells;
% e.g., a random 10% of grassland cells will get 10 seeds, all other cells zero;
```

```
% the matrix 'seeddistributiongrass' will contain seed numbers in each cell of the 'grass' habitat;
doelbiotope = zeros(biotopeR, biotopeC); % a temporary matrix to help determine if species will be
%present in cells;
seeddistributiongrass = zeros(biotopeR,biotopeC);
for i = 1:biotopeR;
   for j=1:biotopeC;
      if biotope(i,j)==1 % 1 = permeability coefficient of target biotope 'grassland'; % if habitat is
%'grassland', set probability of species presence to 'freqgrass';
         targetbiotope(i,j)=rand;
      else targetbiotope(i,j)=100;
      end;
   end;
end;
seeddistributiongrass = (targetbiotope <= freqgrass).*(abungrass.*seedprod); % determine where the
%species is present in 'grass' and assign seed numbers;

% repeat for 'forest' habitat;
targetbiotope = zeros(biotopeR, biotopeC);
seeddistributionforest = zeros(biotopeR,biotopeC);
for i = 1:biotopeR;
   for j=1:biotopeC;
      if biotope(i,j)== 0.394 % =target biotope forest;
         targetbiotope(i,j)=rand;
      else targetbiotope(i,j)=100;
      end;
   end;
end;
seeddistributionforest = (targetbiotope <= freqforest).*(abunforest.*seedprod);

% repeat for 'shrub' habitat;
targetbiotope = zeros(biotopeR, biotopeC);
seeddistributionshrub = zeros(biotopeR,biotopeC);
for i = 1:biotopeR;
   for j=1:biotopeC;
      if biotope(i,j)==0.263 % =targetbiotope shrub;
         targetbiotope(i,j)=rand;
      else targetbiotope(i,j)=100;
      end;
   end;
end;
seeddistributionshrub = (targetbiotope <= freqshrub).*(abunshrub.*seedprod);

% repeat for 'tallherb' habitat;
targetbiotope = zeros(biotopeR, biotopeC);
seeddistributiontallherb = zeros(biotopeR,biotopeC);
for i = 1:biotopeR;
   for j=1:biotopeC;
      if biotope(i,j)==0.386 % =targetbiotope tall herbage;
         targetbiotope(i,j)=rand;
      else targetbiotope(i,j)=100;
```

```
    end;
  end;
end;
seeddistributiontallherb = (targetbiotope <= freqtallherb).*(abuntallherb.*seedprod);

% final seeddistribution matrix, with seed numbers in each cell (all biotopes summed);
seeddistribution = zeros(biotopeR,biotopeC);
seeddistribution =seeddistributiongrass+ seeddistributionforest+ seeddistributionshrub+
seeddistributiontallherb;


% Introduction of large herbivore somewhere inside fence
perimeter = biotope > 0; % area in which animal can move
posR = round(rand*biotopeR); % select random initial row position
posC = round(rand*biotopeC); % select random initial column position

%this loop prevents the animal from starting outside the fence;
while perimeter(posR,posC) == 0;  % if permeability of initial position is zero (outside fence)
  posR = round(rand*biotopeR); % select a new  random initial row position
  posC = round(rand*biotopeC); % select a new random initial column position
end;

% ***modelling of large herbivore movement using a 'constrained random walk'
% simultaneous simulation of attachment and detachment of seeds

% use parameters for focal plant species
adhesion = 0.028; % this is the adhesivity score of seeds on horse fur, see chapter 4
timesteps = 1000; % number of time steps of 15 min
seedload = zeros(timesteps,1);
seedloadT=zeros(timesteps,timesteps+1);
seedload100 = zeros(timesteps, timesteps+1);
seedload100T=zeros(timesteps,timesteps+1);

% the set of parameters (b1, y0) refers to the exponential seed retention curve (with no constant:
%y0=0);
b1 = 0.652; % from % = y0 + 100*exp(-b1*t); % estimated b value from chapter 5;
y0 = 0;

deltat = 0.25;  % fractions of an hour, 0.25 equals 15 min

for t = 1:timesteps % run model through time

% create logfile (containing all information about each timestep)
logpos(t,1)=t; % timestep
logpos(t,2)= posR; % row position of animal
logpos(t,3)= posC; % column position of animal
logpos(t,4)= biotope(posR,posC); % biotope type at position of animal
logpos(t,5)= seeddistribution(posR,posC); % number of seeds in cell in which animal is positioned
```

```
% module to attach seeds to animal
    if seeddistribution(posR,posC) > 0 % if seeds present in cell in which animal is positioned
       seedload(t,1) = (seeddistribution(posR,posC)*adhesion); % put seeds on animal (a fraction of the
      %accessible seed number, defined by the parameter 'adhesion')

       seedload100(t,t) = 1;
        % adjust seed number in cell where animal has passed
       seeddistribution(posR,posC)= seeddistribution(posR,posC) –
       round(seeddistribution(posR,posC)*adhesion); % subtract seeds stuck to animal
    else seeddistribution(posR,posC) = 0;
    end;

% procedure to model animal movement, while preventing the animal from leaving the study area
% use random direction and distance for animal movement;
    distance = animalmovement(round((animalmovementR-1)*rand)+1,1); % select a random distance
    %from distribution of animal movement
    dir=2*pi*rand; % select a random direction;
    posRn = posR + round(distance*cos(dir)); % calculate displacement in x direction
    posCn = posC + round(distance*sin(dir)); % calculate displacement in y direction

 % procedure to adjust direction in function of permeability and to keep animal inside fence
     randomnumber = rand; % select a random number between 0 and 1, called 'randomnumber'

% if animal leaves the landscape or if new biotope has lower permeability than random number
    while (biotopeR-posRn)*(posRn-1) <0 | (biotopeC-posCn)*(posCn-1)<0 | (biotope(posRn,posCn) -
randomnumber) < 0;
        dir=2*pi*rand; % pick a new random direction;
        posRn = posR + round(distance*cos(dir)); % calculate displacement in x direction
        posCn = posC + round(distance*sin(dir)); % calculate displacement in y direction
        randomnumber = rand;
    end;
    posR = posRn;
    posC = posCn;

% calculate proportion of seeds remaining on animal;
% the 't + 80' makes sure that when the mode with constant is used seeds don't remain on forever;
% when the no-constant model is used, the number of seeds is zero anyway after 80 timesteps;
if seedload100(t,t)>0
   seedload100(t,(t+1):min(t+80,size(seedload100,2))) = (y0 + (seedload100(t,t)-y0)*exp(-
b1*deltat*[1:min(80,(size(seedload100,2)-t))]))/100;
end

   t =t+1;
end;

seedload100T=seedload100;

for i = 1:timesteps; % final seed load on animal
    seedloadT(i,:) = round(seedload100T(i,:).*seedload(i,1));
    i= i+1;
```

```
end;

% add distances to logfile (Euclidean distances between each sequential position)
for i=1:timesteps-1;
   logpos(i,7) = round(sqrt((logpos(i+1,2)-logpos(i,2))^2 + (logpos(i+1,3)-logpos(i,3))^2)*10);
% = formula of Euclidean distance
   i=i+1;
end;

% add summed seedloads to logfile (number of seeds on animal at each timestep (summed across
initial %sources))
for i =1:timesteps;
   logpos(i,6) = sum(seedloadT(:,i)');
   i=i+1;
end;

%graphical presentations
subplot(3,1,1), contourf(biotope);
axis([1 biotopeR 1 biotopeC]);
subplot(3,1,2), plot(logpos(:,3),logpos(:,2));
axis([1 biotopeR 1 biotopeC]);
subplot(3,1,3), plot(logpos(:,1),logpos(:,6));
sum(logpos(:,6))/timesteps
mean_seed_load=mean(logpos(:,6))
std_seed_load=std(logpos(:,6))
[mean_seed_load std_seed_load]
```

Chapter 7

Conclusions

This thesis compiles results from observational, experimental and modelling studies of the dispersal of plant seeds by animals (mainly by epizoochory). As study objects, we chose large herbivores used in nature management, as they (1) can serve as models for wild mammals in the present, past and future, (2) are relatively easy to examine, and (3) play a major role in contemporary nature management in our regions.

The main objectives of this study were:

- to identify plant species dispersed by large herbivores
- to quantify seed dispersal by large herbivores
- to compare epi- and endozoochory by large herbivores
- to quantify the adhesive interaction between seeds and furs
- to measure epizoochorous dispersal distances in the field
- to integrate observational and experimental data into a simulation model of epizoochory

In the next paragraphs, the main results -in relation to the above objectives- are outlined and discussed, with special attention to the role of large herbivores as seed dispersers in the context of nature management.

Main findings of this study

A wide range of plant species can disperse their seeds by epizoochory (chapters 2 and 3). Contrarily to what was traditionally assumed (van der Pijl 1982, Grime 1988), epizoochory is not confined to seeds with morphological adaptations for adhesion in fur (e.g. hooks, bristles). This was suggested by the epizoochorous dispersal potential of various seed types (experimentally defined by means of adhesivity scores of seeds to animal furs, see chapter 4), and demonstated by the broad range of seed morphologies observed in the fur of large herbivores in Flemish nature reserves (chapters 2 and 3). Specialized seed adaptations (awns, hooks, bristles, hairs) facilitate adhesion to fur (chapter 4), but are no prerequisite for epizoochory (chapters 2 and 3). Contrastingly, unspecialized seed appendages, such as stems, may even hamper seed attachment to fur (chapter 4). Seeds with adaptations to other dispersal modes, such as hydrochory and anemochory, can also disperse via epizoochory (chapter 2). In our study, we identified 75 plant species of 23 different plant families (the *Poaceae* being overrepresented). Epizoochory seems largely confined to herbaceous species and woody species with non-fleshy fruits (chapters 2 and 3). Although seed size appears -between certain limits- not to be restrictive for epizoochory (both small and large seeds were detected in fur, see chapters 2 and 3), large seeds adhere less well to animal fur than small seeds (chapter 4). The presence of adhesive appendages, however, can counteract the negative impact of seed size on seed adhesivity (chapter 4). On the other hand, if heavy seeds fall on the back of animals with long and undulating furs, their weight may allow them to penetrate deeper into the fur, thus enhancing the retention time in fur (chapter 5). In general, seed morphology is a good predictor of the inherent attachment capacity of seeds in fur (chapter 4). Nevertheless, the observation of many seeds with low adhesivity scores in fur of large herbivores demonstrates that in theory almost all grassland species are able to disperse epizoochorously (chapters 2 and 4).

The species composition of the epizoochorous seed load of animals is related to the availability and the relative abundance of seeds in the local vegetation (chapter 3), which in turn varies with the season (chapters 2 and 3). Nevertheless, also species with low abundances in the local vegetation can be dispersed in animal fur (chapter 3). This can occur in the case of species with very adhesive seeds (chapter 3), but it may also be a consequence of selective habitat use by the animals (cf. chapter 6), or a combination of both.

Epizoochory is only one of two seed dispersal mechanisms mediated by herbivores. Seeds can also be dispersed in dung, after ingestion and passage through the digestive tract of animals (endozoochory). In terms of species composition and trait-based plant types, epi- and endozoochory act on a complementary set of plant species (chapter 3). We observed that epizoochory was more specific than endozoochory, as it was associated with a more narrow range of dispersal-functional plant types. Thus, both epi- and endozoochory are important, as additive and complementary dispersal mechanisms (chapter 3).

Animal behaviour can impact on epizoochory in different ways. As mentioned above, the habitat use of an animal determines the plant species it can come into contact with. Apart from site-specific determinants of habitat use -such as the vegetation type (see chapter 6) and the location of sheltered places and water (Andrew 1988)- also additional factors, such as the animal species (Lamoot et al. 2005) and the composition of the herd (Conradt et al. 1999, 2000) affect habitat use. Even within frequently used habitats, animals may show preference for certain palatable plant species (see Cosyns et al. 2001). Hence, plants could increase the probability of contact with seed dispersing animals by offering a food source (e.g. many graminoid species). Although this has been postulated in the context of endozoochory (‘foliage is the fruit’ hypothesis, cf. Janzen 1984), it may equally apply to epizoochory (chapters 2 and 3). Also other behavioural aspects, such as grooming and rubbing, affect epizoochory (Kiviniemi 1996). Seeds of tall herbs are expected to reach the fur of large herbivores more easily than seeds of small species (cf. Fischer et al. 1996), yet the latter can be picked up by lying or wallowing animals (chapter 2).

Fur traits play a major role in epizoochory (chapters 4 and 5). Deep furs with long, rough, vertically implanted, undulated hairs (Galloway cattle, sheep, wild boar) are better suited for seed adhesion than shallow furs with short, smooth, horizontally implanted, straight hairs (roe deer, Holstein cattle, rabbit, horse). However, an interaction-effect between certain seed and fur types was noticed (chapters 4 and 5). Unappendaged seeds, for instance, adhered disproportionately well to the fur of wild boar, as they could fall more easily between the long, bristly hairs covering its undercoat (chapter 4, see also Mrotzek et al. 1999). Large seeds were retained relatively better in long fur than in short fur (chapter 5). For seed attachment and seed retention, fur type seems more important than animal species (e.g. fur of Holstein cattle is similar to horse fur, but differs greatly from fur of Galloway cattle, see chapter 4).

The average retention times of seeds in fur (chapter 5) can be in the order of magnitude of minutes to hours for short-haired animals (e.g. horses), and hours to days in the case of long-haired animals (e.g. Galloway cattle). Associated dispersal distances approximate tens of meters to kilometers, with occasional dispersal distances far exceeding this values (chapters 5 and 6). While in the case of wind dispersal, the least viable seeds (which are lighter) are generally dispersed further (Strykstra et al. 1998b), this is not likely to be the rule for epizoochory (chapter 5). Hence, it is clear that epizoochory is an important long-distance dispersal mechanism (effecting dispersal beyond 100 m, see Cain et al. 2000). The realized seed dispersal distances are in practice limited by home ranges of animals, or, in the case of domesticated large herbivores, by the size of the pastures or nature reserves. For instance, in a fenced isodiametric 80 ha nature reserve (with a diameter of 1.4 km), the modeled average net seed dispersal distance was 200-250 m, as a result of the restricted movement of the grazers (see chapter 6). In some cases, dispersal distances are artificially increased as a result of animal transports, e.g. in the context of seasonal grazing in Flemish nature management (see chapter 2). Large herbivores then function as mobile links between habitats at large distances. Especially for plants without persisting seed banks (chapter 2), such dispersal links between habitats are crucial for colonization. In terms of seed quantities, epizoochory can be considered as a relatively efficient dispersal mechanism (although probably less than endozoochory, see chapter 3). Despite the limitations of the seedling emergence method, we found an average of 32 viable epizoochorous seeds per herbivore (chapter 2). Our model of epizoochory, which integrates animal movement and behaviour, as well as data on local plant characteristics and parameters of epizoochory (chapter 6), illustrated that the continuous attachment and detachment of seeds on animals summes up to considerable quantities per season or per year.

In summary, this thesis reports on several aspects of (epi)zoochory: (1) the identity and quantity of dispersed species, (2) traits of seeds and plants dispersed by epi- and endozoochory, (3) the potential for seed dispersal in present nature management situations in Flanders, (4) the inherent capacity of seeds to attach to mammalian fur, (5) retention times and potential dispersal distances of dispersed seeds, (6) realized epizoochorous dispersal distances in a concrete study site, predicted by a model based on field data of vegetation composition, dispersal parameters and animal behaviour.

Limitations of this study and directions for future research

The investigation of biotic processes, such as seed dispersal, imposes the difficulty of separating the focal process from interfering ones. In the case of zoochory, a major difficulty is the complex behaviour of animals. The fate of (especially epizoochorous) seeds is hard to follow, as the seeds themselves are hard to trace, and because free-ranging animals are difficult to handle. Although we have partly overcome this problem by using domesticated herbivores for our field research (chapters 2, 3 and 5), this choice restricted the range of investigated animal species and might impose limitations on the extrapolation of the results (e.g. of chapter 6) towards wild animals. However, we used robust breeds of large herbivores (Galloway cattle, Iceland pony, Haflinger pony), which have maintained part of the 'primitive' behavioural and morphological characteristics of their extinct ancestors (van Vuure 2003). Therefore, our results may also (partly) apply to the megaherbivores which originally populated our regions. With respect to an extrapolation of the results towards other mammals, it is likely that at least the mechanistics of the seed dispersal process are highly similar between species. The fact that the horse fur and the Galloway fur (chapter 4) represented both extremes of the suitability of seven furs (among which also three wild mammals) for seed attachment, suggests that the contrast between both animals gives a fair indication of the possible range of seed dispersal potential of our native wild mammals. Obviously, the dispersed plant species and the dispersal distances depend on the frequented vegetation types and the home range of the different mammals.

Despite their domestication, the examined herbivores differed in temper and willingness to be examined, pointing to the critical influence of (unpredictable) animal behaviour on the ease of examining zoochory by living animals. In this respect, further research may focus on the specific impact of animal behaviour -such as grooming, wallowing and rubbing- on seed dispersal. Sorensen (1986) and Kiviniemi (1996) did approach this issue experimentally, yet comparative studies including different animal species are still generally lacking. Also other factors possibly influencing the retention or the loss of seeds from animal fur deserve more attention.

Experimental approaches such as those adopted in this study (chapter 4, 5) allowed the separation of interfering processes and offered the advantage of an increased scientific control. By using prepared furs, we could extend our focus towards wild animals such as roe deer, wild boar and rabbit (chapter 4), and by using marked seeds we overcame the problem of seed traceability in measuring seed retention times in fur (chapter 5). On the other hand, experimental approaches imply the risk of an oversimplification and an incomplete or incorrect reintegration of the different factors influencing dispersal. The experimental methodology may also influence the outcome of the study. For instance, prepared furs (chapter 4) and commercially harvested and dyed seeds (chapter 5) may not be perfect representatives for natural seeds and furs. Fischer et al. (1996), who also used marked seeds for retention experiments in the fur of sheep, avoided changes in the surface structure of the seeds by using small amounts of paint and by leaving rough parts of the seeds without marker. However, they used only two species, with relatively large seeds. Partial seed painting is difficult for small seeds, and may insufficiently enhance seed visibility in fur. Because the range of species size was considerable in our experimental seed mixture (a factor 3.5 for seed length and a factor 87.5 for seed weight, see chapter 5), we assumed that painting all seeds entirely would bias the results less than missing the small seeds. The fact that our seeds were commercially harvested and, as a result, had lost (part of) their adhesive adaptations (e.g. in *Cynoglossum officinale* and *Galium odoratum*), forced us to consider our experimental seeds as abstract species, which were still informative in terms of seed trait - seed retention relationships.

Another methodological limitation is related to the seedling emergence method, which was -as an alternative for direct seed identification- adopted to identify the seeds dispersed in the fur of herbivores (chapters 2 and 3). We showed that this method leads to a substantial underestimation of the quantity of dispersed seeds (chapter 2, see also Stender et al. 1997). Still, the method is easier than direct seed identification, and may still largely overestimate the establishment probabilities of seeds in field conditions, as establishment is another bottleneck in the colonization process (cf. Fig 1.1). In an ecological restoration experiment of British target communities, Pywell et al. (2002) observed that all introduced grass species established, as opposed to 54% to 84% of the sown forb species (non-graminoid herbs). Bischoff (2002) reported that average germination percentages of *Silaum silaus* and *Serratula tinctoria* seeds in petri dishes (66% and 18%, respectively) were approximately twenty-fold higher than in the field (3.1% and 0.2%, respectively). Moreover, after initial germination, more than 50% of the emerged *Silaum* and 25% of the *Serratula* seedlings died during the following two vegetation periods. Kiviniemi & Eriksson (1999) reported establishment percentages ranging from 0% to 25% for 17 grassland species. For certain plant species, the management regime plays a crucial role (Coulson et al. 2001). Establishment of *Rhinanthus* *minor*, for instance, increased by a factor 10 when a hay-cut in July with aftermath grazing was applied. In contrast, *Leucanthemum vulgare* establishment showed no response to different management regimes (Coulson et al. 2001). From the above examples, we can conclude that quantitative estimations of dispersed seeds and species, based on seed germination in laboratory conditions, will generally not underestimate establishment probabilities in field conditions.

Despite the limitations of both observational and experimental research, the field data obtained by these methods (see chapters 3, 4 and 5), permitted us to parameterize a simulation model for epizoochory (see chapter 6), which allowed prediction of observed variations in seed numbers in the fur of animals, as well as realized seed shadows. However, the limited availability of experimental data for some plant species forced us to cross-species utilization of certain parameter values, which may imply a decreased accurateness. In addition, some assumptions with respect to the accessibility of seeds for uptake by passing animals (related to plant height and degree of seed exposure on the plant) had to be made (chapter 6). In fact, there is a general lack of data on the relative accessibility of seeds to seed-dispersing animals. For the assessment of accessible seed numbers, animal behaviour with respect to particular plant species should be considered (e.g. avoidance of some species because of low forage value), as well as determination of actual seed numbers in the field rather than ‘typical’ seed numbers derived from compilations. Reliable data on seed production per plant are only available for very few plant species, and seed production can vary enormously between contrasting habitats (Nathan & Muller-Landau 2000). The further extension of plant trait-based databases, such as the LEDA database (Knevel et al. 2003), may help to integrate and distribute such information.

The potential of epizoochory for ‘directed dispersal’ (disproportionate seed dispersal towards habitats offering relatively high probabilities of survival, cf. Wenny 2001) is also a challenging research issue. Dispersal agents, even within restrictively classified groups, may differ markedly in their effectiveness, both quantitatively (numbers and distances of dispersed seeds) and qualitatively (treatment and deposition of seeds) (Schupp 1993). In this respect, endozoochory is more easy to evaluate than epizoochory, since dung is relatively easy to find in the landscape. Nevertheless, evidence for directed dispersal of epizoochorous plants towards cattle resting sites has been reported (Ernst et al. 1992).

The relationship between epi- and endozoochory in general has received little attention in the literature, probably as a consequence of the mechanistic differences between both processes, and the associated methodological difficulties. For instance, the quantity of epi- and endozoochorous seed dispersal is difficult to compare. In this study, we disregarded the quantitative aspect and focused on the comparison of species and species traits to demonstrate the complementarity of both processes (chapter 3). Yet, many aspects remain unexplored -also the impact of the different post-dispersal environment of epi- and endozoochorously dispersed seeds on seed germination and establishment. Besides dispersal, the post-dispersal fate of seeds is equally important for successful establishment. Comparing the distances travelled and the future fitness of seeds dispersed via zoochory is also an important direction for future research, with important implications for understanding the ecology and evolution of seed dispersal in general. In this context, genetic approaches (e.g. Godoy & Jordano 2001) may be useful in assessing the extent to which plant populations are linked by long-distance dispersal (Cain et al. 2000), yet are in practice only feasible for the comparison between seedlings and potential parents, and can only detect seed movement if it results in successful recruitment (Nathan & Muller-Landau 2000). In conclusion, it is important to investigate the role of both pre-dispersal (e.g. seed production and pre-dispersal seed loss) and post-dispersal (e.g. seed predation, germination, and seedling competition) processes on dispersal patterns (Nathan & Muller-Landeau 2000, Schupp & Fuentes 1995).

Implications for nature conservation

Zoochory and nature management

Dispersal limitation is one of the key factors constraining the colonisation of plants (Turnbull et al. 2000, Zobel et al. 2000, Dalling et al. 2002, Foster & Tillmann 2003, Verheyen et al. 2003), and represents a major bottleneck for the maintenance, development and restoration of target vegetation types (Strykstra 2000, Van Groenendael et al. 1998, Bakker & Berendse 1999, Pywell et al. 2002). Therefore, understanding the mechanisms effecting long-distance seed dispersal is important in the context of nature conservation, management and restoration.

This study shows that large herbivores can mediate the (long-distance) seed dispersal of many more plant species than previously believed on the basis of seed morphology (chapter 2, 3). It seems reasonable to assume that the majority of herbaceous species is inherently capable of dispersal in the fur of herbivores (see chapter 4). Hence, the role of animals in seed dispersal may be more widespread and greater than assumed. Similar seed dispersal mechanisms as we observe must have taken place in the past, when natural populations of large mammals still populated our regions, and regionally migrating livestock herds were common in the landscape. Animals have always been part of our ecosystems (Lundberg & Moberg 2003), and have contributed to the development of our (semi-)natural vegetation types, not only through feeding, trampling and nutrient allocation (Naiman 1988, Olff et al. 1999, Alados et al. 2004), but also through seed dispersal.

Assessing the effect of seed dispersal on the established vegetation is difficult, certainly with respect to epizoochory (but see Ernst et al. 1992), and scientific studies of the impact of seed dispersal on plant recruitment are scarce. The possible impact on vegetation dynamics of endozoochory has been evaluated in the context of heathland conservation and ecological restoration by Welch (1985) and Mouissie (2004). In mixed systems of nutrient-rich and nutrient-poor habitats, a net dispersal of the plant species of nutrient-rich habitats towards the nutrient-poor habitats can be expected (Mouissie 2004). This is a consequence of the herbivores' feeding preference for the more palatable species of nutrient-rich habitats, which thus have a greater chance to be consumed and endozoochorously dispersed than the more unpalatable species of nutrient-poor habitats. Because a net transport of productive species towards low-productive systems conflicts with current conservation goals, Mouissie (2004) recommends not to graze target communities together with nutrient-rich soils (e.g., former arable land) until the nutrient level of the latter has decreased sufficiently. As epizoochory can not be separated from endozoochory, this may be advisable in general. The greater uptake of plant seeds from nutrient-rich habitats might also partly apply to epizoochory, since the herbivores will spend more time in the nutrient-rich habitats. Valuable plant communities in Flanders are often not grazed, so the dispersal of endangered plant species by large herbivores is not likely to happen frequently. In this study (see chapter 2 and 3), we witnessed five ‘red list’ species (Biesbrouck et al. 2001) dispersing epizoochorously (*Anthriscus caucalis, Centaurea nigra, Cynoglossum officinale, Myosotis cespitosa* and *Phleum arenarium*) and five ‘red list’ species dispersing endozoochorously (*Anthriscus caucalis, Galium verum, Koeleria albescens, Leontodon saxatilis* and *Plantago coronopus*). There is no reason to believe that target plant species cannot disperse by zoochory, only there is less opportunity to. Yet, it remains controversial whether the occasional long-distance dispersal of target species by zoochory outweighs the possible negative effects of grazing in general on the target plant communtities (see Welch 1985), especially when only a few small and isolated patches are left. Up till now, the global impact of grazing management on many plant communities is insufficiently understood to allow clear directives.

Currently, there is much debate about active species reintroduction by man (see Van Groenendael et al. 1998, Strykstra 2000). Opponents consider reintroduction an attack on the last stronghold of nature in the communities of semi-natural landscapes, i.e. their species composition, and will only accept it as a last resort to save species from extinction. Others see reintroduction as a useful and legitimate tool in reaching the goals of ecosystem restoration, which can be applied without much hesitation (Strykstra 2000). Van Groenendael (1998) listed some ‘rules of thumb’ with respect to reintroduction: (1) First, habitat conditions should be improved and enough time should be given to allow natural processes of dispersal and establishment to occur (including regeneration from the seedbank) (see also Strykstra et al. 1998a). (2) If this is not successful, natural dispersal can be facilitated by creating seed sources next to the target area or by stimulating seed dispersal vectors. (3) Only as a last possible measure, reintroduction of species can be considered, using seeds from nearby core populations. Van Groenendael et al. (1998) stress the fact that, in fragmented landscapes, species potentially benefitting from reintroduction are species which normally disperse over long distances. Good dispersers will suffer even more from fragmentation -in terms of increased extinction rates and increased inbreeding- when this results in selection pressure against dispersal capacity, an interaction also known as the extinction vortex (Gilpin & Soulé

1986, Lamont & Klinkhamer 1993). Cody & McC. Overton (1996) showed that the evolutionary loss of dispersal-affecting traits may occur within only 10 years!

In many current ecological restoration projects seed dispersal remains the bottleneck, despite improved habitat conditions and attempts to activate regeneration from the soil seedbank. Hence, before considering reintroduction, measures should be taken to restore or improve the functionality of seed dispersal vectors (Van Groenendael et al. 1998). The fact that those plant species which are most likely to benefit from a hypothetical reintroduction after habitat fragmentation, are well-dispersing species (Van Groenendael et al. 1998), highlights the importance of sufficient attention to dispersal vector facilitation, because this measure is likely to operate most on exactly those species.

Given the considerable potential of large herbivores as ‘mobile links’ (see Lundberg & Moberg 2003) between isolated habitat patches by means of seed dispersal (chapter 2, 3, 6), measures should be taken and implemented to restore and increase the animals’ potential home ranges and their possibilities to migrate across our landscapes. The seed dispersal potential of animals should be recognized while outlining nature management measures, as these can have a substantial impact on seed dispersal effectiveness. Given the number of herbivores used for grazing in Flemish nature reserves, the amount of seeds dispersed at a particular moment must be in the order of magnitude of hundreds of thousands in the case of epizoochory (chapter 2, 3) and even more in the case of endozoochory (Cosyns et al. in press a, Mouissie 2004). Although higher seed quantities may be dispersed by endozoochory than by epizoochory (Janzen 1984), both mechanisms act on a complementary set of plant species and plant types (chapter 3), and hence are both important.

To increase the potential of zoochorous long-distance seed dispersal, nature reserves or fenced areas for livestock should preferentially be large and interconnected, either physically or through rotational grazing or seasonal grazing. The effects of habitat fragmentation can be mitigated by the establishment of ecological corridors, as physical connections between habitats (Briers 2002). Such corridors might be more important for the direct migration of animals than of plants (Vandorp et al. 1997, Baum et al. 2004, but see Tewksbury et al. 2002, Kirchner et al. 2003). However, zoochorous dispersal of plant seeds does have a fair chance to take place via movements of animals through ecological corridors. Our results suggest that a wide range of plant species (chapters 2 and 3) can at least potentially be retained long enough in animal fur (chapters 5 and 6) to allow occasional seed dispersal by animals using corridors. Even if only very small amounts of seeds are dispersed over long-distances, this may be extremely important in determining potential rates of plant colonization and plant spread (Higgins & Richardson 1999). Besides plants, it is demonstrated that also other organisms (e.g. insects, spiders and snails) can disperse in mammalian fur (Fischer et al. 1996).

Seasonal grazing is another way of connecting isolated habitats. As a consequence of the limited size of most nature reserves, seasonal grazing -involving regular transports of animals between reserves- is a widely applied nature management measure in Flanders (see chapter 2). The linking of isolated nature reserves through motorized transport of herbivores results in 'accidental' seed dispersal between isolated habitats (chapter 2) and provides a means of 'extra-long'-distance seed dispersal. The herbivores' artificial home ranges and thus the seed movement distances can be increased by up to 200 km in Flanders. Such animal transports may be one of the few remaining 'links' between isolated nature reserves, in terms of genetically connecting plant populations and supplying seeds of 'new species', a prerequisite for colonization. However, also a number of anthropogenic dispersal vectors may link habitats at that scale, especially mowing machinery used in nature reserves (Strykstra 1996, 1997; Couvreur & Hermy 2002). Mowing can cause the dispersal of large numbers of seeds (e.g. up to 24000 germinable seeds by a large machine, see Couvreur & Hermy 2002), yet most species-rich grasslands are usually mown only once or twice a year, while grazing is a more continuous management practice, with large herbivores dispersing seeds during the entire year or vegetation season. Seasonal or rotational grazing may also be manipulated, with the explicit intention to increase seed dispersal from one area to another -by strategically directing the movement of animal herds in space and time from target seed sources towards insufficiently developed vegetation types. However, this is generally not (yet) applied. Reasons are the lack of scientific experience with such practises, the fear of nature managers that 'undesired' rather than 'desired' species will be dispersed among vegetation types and terrain compartments (see Mouissie 2004), and the decision of nature managers not to interfere with biotic processes affecting species compositions (see Strykstra 2000).

Besides by large scale ecological networks and directed grazing, seed dispersal can also be stimulated in a more passive way. The choice of the herbivore species can have a substantial effect on the effectiveness of epizoochory. Especially long-furred animals (see chapter 4, 5), such as Galloway cattle, are suited for this purpose (chapter 5, see also Stender et al. 1997). In addition, herds composed of animals of both sexes are expected to use their habitat more elaborately (‘habitat segregation’, cf. Conradt 1999, 2000) than monotypic herds, and may as such reach more plants as more parts of their home range are explored. An additional advantage of zoochory is that it provides a reasonable chance of directed dispersal, since animals frequent similar habitats and create suitable germination sites by disturbing the established vegetation (Janzen 1984, Stiles 1992, Wenny 2001). An indication of directed dispersal by epizoochory is provided by Ernst et al. (1992), who reported a dominance of epizoochorous species under cattle resting sites in Botswana.

Despite the growing evidence for the necessity of landscape defragmentation, the present situation in Flanders still lacks sufficient dispersal-promoting measures such as a network of habitats and habitat corridors. Attempts to delineate and establish an ecological network (Vlaams Ecologisch Netwerk, VEN) are delayed by short-term economic priorities and political decisions. Still, Watkinson & Gill (2002) emphasized that dispersal and changes in species distribution are likely to become increasingly significant and that present conservation approaches with their emphasis on stasis are likely to prove inadequate in the face of climate change, which will drive the movement of species, the dissociation of communities and changes in habitat structure. In addition, given the considerable time lag (50-100 years) in the response of plant species diversity to changing configuration of habitats in the landscape, restoring former habitat connectivity will not result in a rapid increase of species diversity. More likely, future species loss may be expected even if the present landscape is maintained (cf. the so-called extinction-debt, cf. Lindborg & Eriksson 2004). To mitigate this process in the present western-European landscape, which is characterized by habitat degradation, fragmentation and limitation of natural dispersal processes, it is crucial to restore and enhance dispersal processes (Bakker et al. 1996, Bonn & Poschlod 1998, Poschlod & Bonn 1998, Bakker & Berendse 1999).

We think that domesticated large herbivores can partly adopt the role of extinct large animals and former free-ranging livestock herds in seed dispersal (see also Pykälä 2000, Bruun & Fritsbøger 2002). Consequently, they may be an important tool in ecological restoration projects, where dispersal is a bottleneck. Dispersal-promoting measures, involving animals as ‘mobile links’ (see also Poschlod et al. 1996, Lundberg & Moberg 2003), may facilitate and accelerate vegetation development, particularly in new nature reserves established on former agricultural land (see also Gibson et al. 1987, Briers 2002). The fact that many of the epizoochorously dispersed species lack persistent seedbanks (chapter 2) -in contrast to endozoochory (Pakeman et al. 2002)- is also particularly important in a nature restoration point of view, since the loss of species with short-lived seeds can only be counteracted by successful dispersal. As mobile link organisms, large herbivores may help to mitigate the catastrophic effect of habitat fragmentation and degradation (which resulted in a decreased seed availability in the landscape) and of the disappearance of free-ranging wild animals and migrating herds (which functioned as seed dispersal vectors for plants). Moreover, the herbivores enhance the dispersal opportunities of plants over distances which might be crucial in the face of climate change (see also Watkinson & Gill 2002, Higgins et al. 2003a).

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Curriculum vitae

Martine Couvreur (°Leuven, 21 april 1975) behaalde het diploma hoger secundair onderwijs, optie Wiskunde Wetenschappen, aan het Don Bosco Instituut te Haacht in 1993. In 1997 studeerde ze af als Biologe aan de Faculteit Wetenschappen van de KULeuven, en in 1998 behaalde ze het diploma van de Academische Lerarenopleiding aan de KULeuven. Van oktober 1997 tot september 2001 was ze werkzaam als FWO-aspirante. Tijdens de eerste 2,5 jaar van deze periode was ze verbonden aan het Labo voor Moleculaire Celbiologie (Faculteit Wetenschappen, KULeuven), waar ze onderzoek deed naar glucose-geïnduceerde signaaltransductie bij de gist *Saccharomyces cerevisiae*. Vanaf maart 2000 werkte ze aan het Labo voor Bos, Natuur en Landschap (Faculteit Bio-ingenieurswetenschappen, KULeuven), waar ze onderzoek deed naar de verbreiding van plantenzaden door grote herbivoren (epizoöchorie) en natuurbeheermachines (agestochorie). Na oktober 2001 zette ze dit onderzoek verder als vrijwillig medewerker aan de KULeuven. In die periode werkte ze ook mee aan twee projecten in opdracht van het Ministerie van de Vlaamse Gemeenschap: 'Ecodistricten: Ruimtelijke eenheden voor gebiedsgericht milieubeleid in Vlaanderen' (februari 2002 - september 2002) en 'Ecologische inventarisatie en visievorming in het kader van Integraal Waterbeheer, Stroomgebied van de Bosbeek' (juni 2003, april-mei 2004). Van december 2004 tot februari 2005 was ze als doctoraatsbursaal verbonden aan het Labo voor Bosbouw (Faculteit Bio-ingenieurswetenschappen, UGent).

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Martine 21 maart 2005

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