Fungal Ecology 28 (2017) 25–32

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Fungal diversity on dung of tropical animals in temperate environments: Implications for reconstructing past megafaunal populations

Eline N. van Asperen*

School of Natural Sciences and Psychology, Liverpool John Moores University, UK

ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form
1 December 2016
Accepted 15 December 2016
Available online 16 May 2017

Corresponding Editor: John Dighton

Keywords:
Communities
Coprophilous fungi
Diversity
Megaherbivores
Seasonality

ABSTRACT

Coprophilous fungal spores from sedimentary sequences are increasingly used to reconstruct past herbivore presence and abundance. To provide a modern analogue for extinct megaherbivores in temperate environments, the coprophilous fungal communities on dung of exotic megaherbivores in a temperate environment were compared with those of (semi-)native wild, feral and domesticated herbivores. Six zygomycete, 32 ascomycete and one basidiomycete taxa were identified. A large overlap in community composition was observed between samples from different geographical locations. Dung fungal diversity was influenced primarily by the range size of the herbivore and season of collection. Diet and digestive system were not significant determinants of dung fungal diversity. The dung of exotic megaherbivores is characterised by a dung fungal community similar to that of (semi-)native herbivores, and therefore provides a good analogue for the dung of closely related but now extinct temperate megaherbivores.

1. Introduction

Coprophilous fungi are a diverse group of fungi that grow on animal dung, encompassing genera from most major taxonomic groups (Wicklow, 1992; Richardson, 2001; Krug et al., 2004). Some of these grow almost exclusively on dung, whilst other species also grow on a variety of other substrata. Many exclusively coprophilous species, especially those belonging to zygomycete (Pilobolaceae) or ascomycete genera, release their sporangia or individual spores using a variety of explosive mechanisms (Ingold, 1971; Trail, 2007; Yafetto et al., 2008), propelling them onto the surrounding vegetation. The spores are then ingested along with the vegetation by herbivores, pass through the animal's digestive system and are voided with the dung. Whilst it is unclear whether the passage through the animal's gut plays any role in the germination of these spores, due to their presence on vegetation herbivore dung generally harbours a more diverse fungal community than carnivore dung (Lundqvist, 1972; Furuya, 1990).

Many coprophilous fungal spores (primarily ascomycete) are thick-walled, and often the walls contain pigments which protect the spore from exposure to harmful UV radiation (Lundqvist, 1972). This also accounts for their long-term survival in soils (Van Asperen et al., 2016) and their consequent presence in sedimentary samples. Over the past decades, counts of coprophilous fungal spores from sedimentary sequences have increasingly been used to reconstruct past herbivore presence and abundance (e.g. Davis, 1987; Davis and Shafer, 2006; Baker et al., 2013). In particular, this proxy plays an important role in studies to determine the timing and impact of the worldwide extinction of megaherbivores at the end of the last ice age (Gill et al., 2009; Feranec et al., 2011; Gill, 2014; Johnson et al., 2016). In North America, northern Asia and Europe, the Late Pleistocene extinctions affected species such as elephants (e.g. mammoths and mastodons, as well as the temperate-adapted Eurasian forest elephant) and rhinoceroses (e.g. woolly rhinoceroses, as well as temperate-adapted Eurasian rhinoceroses) whose extant relatives now only survive in tropical regions (Owen-Smith, 1987; Koch and Barnosky, 2006; Stuart, 2015). Furthermore, many other large herbivores such as deer, bovids and horses, also went extinct or suffered severe population and range reductions, which often later resulted in further extinctions or range reductions. After the extinctions, the large herbivore fauna in temperate

* Current address: Department of Biosciences/Department of Anthropology, Durham University, Stockton Road, Durham, DH1 3LE, UK.
E-mail address: envanasperen@palaeo.eu.

http://dx.doi.org/10.1016/j.funeco.2016.12.006
1754-5048/

environments in Europe was dominated by large bovids (aurochs), several deer species and horses (Stuart, 2015).

Most studies of coprophilous fungi in temperate regions have focused on (semi-)wild lagomorphs (rabbits and hares), wild deer or domesticated animals (sheep, cattle and horse; e.g. Harper and Webster, 1964; Richardson, 1972, 2001; Angel and Wicklow, 1975, 1983; Parker, 1979; Morinaga et al., 1980; Wicklow et al., 1980; Piontelli et al., 1981; Caretta et al., 1994; Nyberg and Persson, 2002; Kruys and Ericson, 2008). There are some studies on coprophilous fungi growing on elephant dung in tropical environments (Ebersohn and Eicker, 1992; Masunga et al., 2006; Piasai and Manoch, 2009; Mungai et al., 2011, 2012a, 2012b, 2012c, 2012d, 2012e), but very few studies on rhinoceros dung (Mungai et al., 2012a, 2012b, 2012c). Most coprophilous fungal genera, and many coprophilous species, have a cosmopolitan distribution (Webster, 1970; Richardson, 1972, 2001; Krug et al., 2004), although some species are more common in southern latitudes than in northern latitudes and *vice versa*. Community diversity tends to decrease with increasing latitude, and is at its highest in the tropics (Lundqvist, 1972; Richardson, 2001; Krug et al., 2004). Although the general character of the diet (grazer, browser or mixed feeder) will be similar across the globe for closely related herbivore species, the particular plant species consumed will vary. For these reasons, fungal communities on the dung of tropical animals living in tropical regions may not provide a close analogue for the dung of extinct temperate megaherbivores. To provide a closer analogue, here the coprophilous fungal communities on dung of exotic megaherbivores in a temperate environment are compared with those of (semi-)native wild, feral and domesticated herbivores. This is also the first study of the fungal community occurring on the dung of feral cattle in a temperate environment.

2. Material and methods

Freshly voided dung of African elephant (*Loxodonta africana*, n = 4), white rhinoceros (*Ceratotherium simum*, n = 4), Ankole cattle (an African breed of *Bos taurus*, n = 3) and fallow deer (*Dama dama*, n = 3) was collected in sterilised containers from Knowsley Safari Park (Prescot, UK; see Fig. 1) on 17 September 2014 and 22 April 2015. Except for the elephants, the animals are outside continuously during summer, whereas in winter, they are kept in shelters at night and free to roam the reserves during the day. Reserve sizes and stocking numbers can be found in Table 1. Whilst on the reserves, the animals can graze and browse freely and access to water is provided. They receive a supplement of hay and dry feed consisting of pellets and small amounts of fruit and vegetables throughout the year. Salt licks, mineral licks and molasses are also made available to those species which benefit from this. The Ankole cattle and fallow deer are wormed 2–3 times per year, whilst the elephants and white rhinoceroses are wormed only when nematodes are found in faecal samples. The animals receive other medication, such as antibiotics, only when necessary.

Freshly voided dung of free-ranging feral cattle originating from a local breed of domestic cattle (*B. taurus*, n = 6) was collected from Chillingham Wild Cattle Park (Chillingham, UK) on 5 June 2014, 10 October 2014, 2 December 2014 and 15 April 2015 as a comparison for the African breed of cattle (see Table 1). A feral herd of Chillingham cattle has lived in this area since at least 1646 without any human handling or veterinary intervention, apart from occasional culling of aged or diseased animals (Hall, 2007, 2013). In winter, limited supplementary hay harvested locally and compound feed is provided if necessary, and in previous years, limestone has been applied to the grazing areas to prevent dietary magnesium deficiency (Hall et al., 2005). To control for geographical variation, freshly voided dung of free-ranging fallow deer (*D. dama*, n = 5) was also collected from Chillingham Wild Cattle Park on 5 June 2014, 10 October 2014 and 15 April 2015. As a comparison for the hindgut-fermenting elephants and rhinoceroses, freshly voided dung of a domestic horse (*Equus caballus*, n = 2) was collected from Stevenage (UK) on 12 May 2016. The horse was fed primarily hay with some supplementary feed, and grazed in pasture. It received anthelmintic and other veterinary treatment when necessary.

The use of anthelmintics can have an adverse effect on dung-inhabiting organisms such as beetles, collembolans and fly larvae (Römbke et al., 2010; Beynon, 2012; Lumaret et al., 2012). It is unknown whether these chemicals also affect dung fungi. Since the adverse effects on other dung-inhabiting organisms tend to wear off within about a week after the drugs are administered (Römbke et al., 2010; Beynon, 2012; Lumaret et al., 2012), samples were taken at least 20 d after the last treatment.

Knowsley and Chillingham samples taken on the same date represent different individuals. It cannot be excluded that samples taken on different dates stem from the same animal, although this is unlikely for Chillingham, where about 100 cattle and 150 fallow deer are present. The horse samples were collected from a single individual. The April, May and December samples represent winter/spring diets, whilst the June, September and October samples represent summer/autumn diets. The samples were stored for 2 d in the dark at 4 °C. 10–60 g of each sample of fallow deer dung and 100–200 g of each sample of dung of the other species was placed on moist paper towels in sterilised glass dishes with glass lids and incubated for 60–80 d at 20 °C under natural light conditions (~12 h of daylight d^{-1}; Krug, 2004). The samples were kept moist by periodically wetting the paper towel with distilled water. One sample of horse dung was split into two subsamples, one of which was incubated under standard laboratory conditions, the other in the dark at 4 °C.

During the incubation period, the dung samples were examined every 3–4 d using a stereomicroscope. Spore-producing fruit bodies growing on the dung were mounted in alcohol and lactophenol cotton blue, and identified under a light microscope. Measurements of the fruit bodies and spores present were taken to aid in identification.

Similarity in the species composition between the different substrates, as well as between the Knowsley and Chillingham samples as a group and between winter and summer samples, was calculated using the Sørensen-Dice coefficient (Dice, 1945; Sørensen, 1948), defined as $SD = 2c/(a+b)*100$, where a = the number of species found on one substrate; b = the number of species found on a second type of substrate, and c = the number of species common to both.

Estimates of total species richness were made using $Chao_2$ (Colwell and Coddington, 1994), the first-order jackknife (Heltshe and Forrester, 1983) and the second-order jackknife (Smith and Van Belle, 1984). These are defined as:

$$Chao_2 = S_{obs} + (L^2/2M)$$

$$Jackknife\ 1 = S_{obs} + ((n-1)/n)L$$

$$Jackknife\ 2 = S_{obs} + L(2n-3)/n\text{-}M(n-2)^2/(n^2\text{-}n),$$

where S_{obs} is the number of species observed, n = number of samples, L is the number of species recorded only once in the sample set and M is the number of species recorded only twice in the sample set. Sample size has a relatively strong influence on these estimates, but the larger the number of samples, the more robust the estimate (Colwell and Coddington, 1994).