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Faeces of generalist predators as 'biodiversity capsules': A new tool for biodiversity assessment in remote and inaccessible habitats

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ABSTRACT

Molecular methods are increasingly used to identify prey DNA in predators' faeces to describe diet composition. However, such analysis can reveal much more ecological information. If faeces are regarded as 'biodiversity capsules', they can help describe and quantify ecological communities by containing a representative sample of the prey species occurring in the foraging area of a given predator. Here we propose to analyse these 'capsules' and infer the occurrence, distribution and minimum abundance estimate of prey communities. This novel approach goes beyond the detection of 'targeted' prey groups to inform dietary studies of predators. It is particularly suited to the study of prey communities that are difficult to sample with traditional methods because they are very small, rare and/or live in remote or inaccessible habitats. Such communities include invertebrates inhabiting the soil, deep-sea species, and small, rare flying insects. The proposed approach has the potential to inform the topical issue of biodiversity assessment and provide a new framework for the discovery of species with minimum interference to ecosystems and without the need for extensive trapping, which can be labour intensive and could kill many individuals of non-target species. Rigorous testing of this approach, and in particular direct comparison with traditional sampling methods is required to fully demonstrate its efficacy.

1. Introduction

In the past decade molecular techniques have been increasingly used to detect prey DNA and identify predated species using gut contents or faecal samples from predators (Symondson, 2002; King et al., 2008; Symondson and Harwood, 2014). For predators of no conservation concern, individuals can be sacrificed and prey tissue in the guts can be isolated and used for visual or DNA analysis (Carreon-Martinez et al., 2011). However, it is not always possible to detect and isolate such tissues from the gut contents of predators. This is the case for many predatory species, including those feeding on prey which are mainly soft-bodied such as the juvenile stages of many holometabolous insects (45% of all animal species), molluscs (85,000 known species), earthworms (up to 98% of all animal biomass in forest ecosystems (Brockie and Moeed, 1986)), etc. Similarly, the detection of prey tissue samples is not possible in the gut of liquid-feeding predators such as spiders, true bugs (Hemiptera) and mites. The analysis of faecal DNA samples is more widely applicable than morphological studies but suffers from particular technical limitations. One major issue has been the presence of mixed DNA from several prey species in one faecal sample, which causes standard (Sanger dideoxy) DNA sequencing to fail (Boyer et al., 2011). Although this is not an issue for species with highly specialised diets (Rougerie et al., 2011), this is particularly limiting for generalist predators that feed on a variety of prey species. Until recently, such mixed DNA samples could be analysed only after the development of large panels of species-specific primers (Jarman et al., 2004) used in complex multiplex PCRs (Harper et al., 2005; King et al., 2011) or cloning analyses (Zeale et al., 2011).

By combining (i) high-throughput next-generation sequencing (e.g. Margulies et al., 2005), (ii) the use of individual molecular tags (Parameswaran et al., 2007), and (iii) new bioinformatic tools for the selection of mini-barcodes (e.g. Brown et al., 2012), it is now possible to sequence mixed DNA samples and identify every species present in the diet of a given predator (Boyer et al., 2012). Accurate identification at the species level remains dependent on the comprehensiveness of the available DNA sequence reference libraries and the capacity of group-specific primers to amplify a mixture of DNA. Beyond the implications for conservation biology, particularly in endangered predatory species for which feeding behaviour is difficult to observe, combining these recent molecular techniques provides a new tool for rapid and easy assessment of animal communities through predator faeces. The latter can therefore be regarded as 'biodiversity capsules' possibly containing a representative sample of prey species occurring in the

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predator's foraging area. Although other sources of environmental DNA have been used for such purpose (Box 1), faecal samples have rarely been considered in such studies.

2. Estimates of prey species diversity using predator faecal samples

Developing novel tools to better assess biodiversity is crucial to the discovery of new and cryptic species, the study of rare and poorly known taxa, and the monitoring and conservation of endangered and declining species, all of which are key to better understanding and addressing the current biodiversity crisis (Barnosky et al., 2011). With new species documented every day (Zhang, 2011), many may disappear even before they are discovered (Barnosky et al., 2011). Some are very difficult to detect or study because they are very small and/or at a low density in remote or impenetrable habitats such as those designated as main biotic frontiers (André et al. 1994). This includes, but is not limited to, soil invertebrates, species inhabiting deep seas and small and rare flying insects. Generalist predators feeding on such species essentially harvest their DNA (Fig. 1), which can then be readily retrieved from faecal samples and used as proxy for prey species inventories and ecosystem monitoring programmes (Jarman et al., 2013). With a comprehensive network of known collection sites and basic knowledge about the predator's dispersal abilities, prey occurrence data could also be used to draw distribution maps. This may require cautious correction for potential methodological biases such as prey preferences, detection limit, PCR bias etc. (see the Strategies and limitations section).

Because predators have evolved to find their prey efficiently, their faeces can contain prey species that would have been difficult to collect using conventional sampling methods. For example, Bohmann et al. (2011) analysed 89 faecal pellets to assess the diet of two African bat species (Chiroptera: Molossidae) and found DNA from 236 different prey taxa (molecular operational taxonomic units, MOTUs). Almost 80% of these were detected in only one guano pellet. In another study by Burgar et al. (2014) 190 MOTUs were detected from 63 faecal samples produced by three different bat species, but only 20% of these MOTUs could be assigned to known species. Although the authors did not identify taxa at the species level, it is likely that many of these MOTUs were rare or even unknown species and they would have been difficult to collect and identify using conventional sampling methods without considerable trapping effort. Similar patterns have been observed for terrestrial predators that feed on marine prey but produce faeces on land. In 2007, Deagle et al. (2007) studied the diet of Macaroni penguins (Sphenisciformes: Spheniscidae) using faecal samples. Among the six species of fish for which 16S mtDNA sequences were retrieved there was one that did not match any sequence in GenBank and was about 20% divergent from any other fish species for which 16S sequences were available. This species could not be classified further than to the superorder of Acanthopterygii. Despite the great interest and comprehensive body of knowledge existing for this group, and very high rates of DNA sequencing in the recent years (Ward et al., 2009; Becker et al., 2011; Trivedi et al., 2015), the unidentified species still displayed 16% divergence from any other fish species sequenced to date. These sequences are therefore likely to correspond to yet undescribed species, which illustrates the potential of faecal samples to inform future sampling efforts and support the discovery of new species. Indeed, the discovery of new DNA sequences that do not match any known species gives us a clue with regard to the distribution of these potential new species. If they are only found in faeces collected at a certain time of the year or at a certain location, this gives indications of where and when to focus sampling efforts to collect specimens necessary for formal species discovery.

Another similar example is given by Deagle et al. (2009) who analysed the diet of Australian fur seals (Carnivora: Otariidae) and found 15 fish species (27% of the predated species) for which classification could be determined only to family (1), order (2), superorder (2) or infraclass (10) level. Even sampling programmes that appear to be

Box 1
Assessing biodiversity using other sources of environmental DNA.

Environmental samples are often easier to collect than are individuals themselves. This is not limited to faecal samples; other sources of environmental DNA have also been used to assess biodiversity, with successes and limitations. For example, environmental DNA from water samples has been used to assess vertebrate and invertebrate biodiversity in stagnant and running-water ecosystems (Ficetola et al., 2008; Goldberg et al., 2011; Hajibabaei et al., 2011). Thomsen et al. (2012) proposed a simple model that estimates population abundance based on animal body size and DNA degradation rate, for two amphibian species in a closed freshwater system and under controlled conditions. This study provides a first insight in estimating species abundance in freshwater systems. Although small animals can be detected in large amounts of closed water (e.g. 0.08 g fish per litre (Collins et al., 2012)), one important drawback is the rapid decrease in DNA concentration through time, especially in running-water systems (Dejean et al., 2011). In marine environments, water samples have mostly been used to study microbial communities (Zinger et al., 2012); however, the analysis of sediment samples has demonstrated the possibility of creating biodiversity inventories of eukaryotes at broad taxonomic scales (Pawlowski et al., 2011).
Soil DNA samples (sometimes called 'dirt' DNA) have been used by Andersen et al. (2012) as indicators of vertebrate diversity in zoological parks. However, this study focused on large vertebrates (elephants, ostriches, lions, giraffes, etc.) for which detection by conventional visual techniques is often more appropriate and environmental DNA sampling rarely necessary. The biomass of animal populations at a given site appeared to be one of the main drivers of DNA detection in soil samples (Andersen et al., 2012), suggesting that this approach would be considerably less effective for detecting smaller and/or less abundant species. Bienert et al. (2012) proposed a method for detecting the DNA of invertebrates from soil samples and identified nine co-occurring species of earthworms. This method appears sensitive enough to detect small quantities of extracellular DNA (possibly deriving from excreted fluids (Minamiya et al., 2011), faeces (Lefort et al., 2012) or exuviae (Lefort et al., 2012) of invertebrate species) and is therefore promising for biodiversity inventories. However, Bienert et al. (2012) did not discuss the possibility of estimating species abundance. One issue with their proposed method is that large quantities of DNA from one live specimen accidentally taken in one of their 0.5 kg soil cores could dominate the PCR process (Deagle and Tollit, 2007) and mask the presence of other species, or at least result in a biased representation of species abundance. Such bias could be minimised by collecting several small soil cores and mixing them together to make a more representative sample (Taberlet et al., 2012).
Another example is DNA from regurgitation pellets that are produced by birds of prey and contain remains of prey that could not be digested. Because individual fragments (bones, feathers, fur, teeth etc.) can be easily isolated from pellets and analysed individually, molecular analysis of these does not involve mixed DNA samples and can be performed using Sanger sequencing (Taberlet and Fumagalli, 1996). Such analyses have been performed to investigate the diversity and genetic variability in small mammals inhabiting the foraging areas of raptors (Hadly et al., 2003) and barn owls (Poulakakis, 2005). Although this method can potentially inform biodiversity assessment of prey communities and shares similarities with the biodiversity capsule approach, it appears to be much more limited. Indeed, DNA from digestion pellets is only applicable to certain predatory bird species, and cannot be applied to soft-bodied prey. These methods are therefore much more limited and unlikely to produce as much insight as the analysis of faecal samples.