SHORT COMMUNICATION

Genetic identification of mammalian meal source in dung beetle gut contents

Andrés Gómez a and Sergios-Orestis Kolokotronis b

a ICF International, Washington, DC, USA; b Department of Biological Sciences, Fordham University, Bronx, NY, USA

ABSTRACT

Coprophagous dung beetles are a numerically and functionally important group. Their obligatory use of mammalian dung has broad ecological implications, including providing economically and epidemiologically relevant ecosystem services. Beetle-mammal ecological networks are critically important in determining the resilience of dung beetle communities and the supply of beetle-mediated ecosystem functions. However, our understanding of dung beetle trophic networks remains incomplete. Here we report on a pilot study to evaluate the effectiveness of DNA-based analyses in identifying the source of dung beetle meals. Using beetles collected from dung piles of known provenance, we hypothesized that molecular analysis of gut content would correctly identify the mammal host, and that beetle body size would increase the odds of successful detection of mammalian DNA. We analyzed 90 specimens belonging to six beetle species. Most samples yielded mtDNA sequences from the expected mammalian species, suggesting that these methods can be an efficient tool for the investigation of dung beetle diet.

Introduction

Mapping the structure of ecological networks is a critical step in understanding ecosystem functions and their likely responses to disturbance. Insights into network structure and dynamics are essential for answering a broad suite of ecological and evolutionary questions, and for designing and implementing strategies to conserve biodiversity (Evans et al. 2013). Unsurprisingly, this is an area of active development, and novel empirical and theoretical research has greatly increased our understanding of ecosystem architecture (Bascompte 2010; Ulanowicz et al. 2014).

Coprophagous dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) have obligatory associations with mammal dung. Adult dung beetles use mammal feces in reproduction, relocating dung balls in which they lay their eggs. Dung is also their main source of nutrition and dung beetles use specialized mouth parts to process feces prior to ingestion (Holter & Scholtz 2007). The consumption and relocation of dung has broad ecological implications. Dung beetles perform important ecosystem functions and provide economically and epidemiologically relevant ecosystem services through their contributions to nutrient cycling, seed dispersal, soil aeration, and parasite and pest control (Nichols et al. 2008; Nichols & Gómez 2014). Dung beetles appear to be particularly sensitive to ecosystem alteration, sharply declining in richness, abundance, and evenness following environmental disturbance (Nichols et al. 2007). These responses may be mediated by changes in the composition and availability of the dung supply through alterations in the abundance and composition of mammal communities (Nichols et al. 2009).

Dung beetle resource use creates a network of linkages between beetles and mammals, and this ecological network also includes mammal parasite species with a fecal life stage. These beetle-parasite ecological linkages have potentially important epidemiological consequences (Nichols & Gómez 2014), and are ultimately mediated by the beetles’ preferences for mammalian dung. Detailed knowledge of beetle feeding in nature is thus basal to our understanding of these ecological networks. In turn, understanding the beetle-mammal linkages is critically important in determining the resilience of dung beetle communities and the supply of beetle-mediated ecosystem functions. More eloquent treatments of dung beetle ecological functions and networks have been provided by Nichols et al. (2007, 2008, 2009).

Despite of over a century of natural history research on dung beetles, their feeding biology is not well understood (Holter et al. 2002; Holter & Scholtz 2007). The ephemeral and patchy nature of dung suggests that strict dietary specialization in dung beetles should be rare (Hanski & Cambefort 1991). Therefore, dung beetles are generally assumed to be polyphagic and to show only guild-level preferences for dung (Dormont et al. 2004). However, extreme dietary specialization is possible (Jacobs et al. 2008), and preferences can range from mostly indiscriminate to more specialized associations (Fincher et al. 1970; Tshikae et al. 2008). Further, some species appear to show strong geographical variation in dung preference and others have been observed in association with dung types previously assumed to be outside their preferred food sources (Cave 2005; Dormont et al. 2004).

Recent studies have used molecular analyses to identify and quantify the strength of ecological interactions in trophic
networks (Alberdi et al. 2012; Wirta et al. 2014). Molecular analyses using samples derived from associate species have yielded important insights about their feeding ecology and the composition of the host community (Calvignac-Spencer et al. 2013). These methods are inexpensive and are sensitive, accurate, and can help circumvent some of the limitations of other research tools (Galan et al. 2012). Here we report on a pilot study to evaluate the effectiveness of DNA-based analyses in identifying the source of dung beetle meals using gut contents. Using beetles known to have been feeding or attempting to feed on a dung pile of known provenance, we hypothesized that DNA sequence analysis of gut content would correctly identify the species of mammal (hereafter, the host), and that beetle body size would be a predictor of the likelihood of successful detection of mammalian DNA.

Materials and methods

Field collection

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae and Aphodinae) were sampled using the floatation method (Moore 1954) from 25 horse dung pats, 24–48 h in age, between 8 and 10 September 2013. The collection site was a privately owned horse farm, in a temperate broadleaf deciduous forest region of central New Jersey, USA (40.2° N, 74.6° W). Collected individuals were stored in 95% v/v ethanol at −20°C. All individuals were identified to species level using standard taxonomic keys and measured along their main axis.

Dissection

To minimize the risk of contamination with mammalian DNA from dung adhering to the beetle’s exoskeleton, we dissected all beetles using a three-step protocol. A dedicated dissection kit was used to carry out each part of the dissection. First, beetles were sequentially rinsed vigorously in distilled water, 70% v/v ethanol, and water again. Any visible dung remnants were removed at this stage. Second, the abdomen was accessed through a dorsal approach by removing the elytra, wings, and abdominal tergites. Finally, a second dissection kit was used to carefully remove the hemocoel contents, which were stored in 95% v/v ethanol and stored at −20°C. Dissection kits were thoroughly cleaned after each dissection by wiping with paper towels, rinsing in alcohol, and exposing to a flame until red after each individual dissection, thereby destroying any DNA that may have originated from the external surfaces of each beetle or from hemocoel contents from previously dissected beetles.

Laboratory and analytical procedures

Following dissection, the intestinal tract of individual dung beetles was examined under a dissection microscope and excised to isolate intestinal contents whenever these were visible. It was not possible to clearly identify gut contents from small-bodied beetles, and in these species the complete hemocoel contents were used for analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean length (mm)</th>
<th>Range</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphodiopsis ruscicola</td>
<td>4.8</td>
<td>4.4-5.2</td>
<td>10</td>
</tr>
<tr>
<td>Copris minutus</td>
<td>9.8</td>
<td>8.5-10.3</td>
<td>4</td>
</tr>
<tr>
<td>Geotrupes blackbarnii</td>
<td>20.1</td>
<td>18.8-21.4</td>
<td>2</td>
</tr>
<tr>
<td>Onthophagus hecate</td>
<td>7.1</td>
<td>6.2-8.5</td>
<td>22</td>
</tr>
<tr>
<td>Onthophagus orpheus</td>
<td>5.3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Onthophagus pennsylvaniae</td>
<td>4.6</td>
<td>3.7-5.6</td>
<td>14</td>
</tr>
<tr>
<td>Onthophagus taurus</td>
<td>9.4</td>
<td>7.0-11.6</td>
<td>37</td>
</tr>
</tbody>
</table>

These samples were used as template for DNA extraction that was carried out using the DNeasy Blood & Tissue kit (QIAGEN) as per the manufacturer’s protocol with the modification of incubating the intestinal contents (i.e. fecal pellets) in 180 µl of ATL buffer and 20 µl of Proteinase K at 56°C overnight. Given that the target DNA located in the horse intestinal epithelial cells was expected to be very degraded, since it was in the fecal product of digestion of horse dung by the beetles, we aimed at PCR-amplifying mitochondrial DNA fragments measuring less than 200 bp. For that purpose we selected the Control Region, or D-loop, locus using oligonucleotide primers previously developed for ancient DNA and more specifically DNA extracted from equid bones dating up to the late Pleistocene (Vilà et al. 2001). The most successful pair of PCR primers from Vilà et al. (2001) was pair 2 (renamed here as Horse_CR_B1: 5'-CCCACCTGACATGCAATAT-3' and Horse_CR_B2: 5'-TGTTGACTGGAAATGATTTG-3'). PCR was carried out using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare), 0.5 µl of each primer, and 1.5 µl of template genomic DNA. The thermal cycling conditions were as such: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. PCR-positive samples were purified using ExoSAP-IT (Affymetrix) and bidirectionally sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Inc. [ABI], Life Technologies) using BigDye v3.1 chemistry. Nucleotide sequences of the hypervariable region 1 (HVR1) portion of the D-loop were matched against the nr database of NCBI GenBank using MegaBLAST (http://blast.ncbi.nlm.nih.gov).

Results and discussion

We analyzed 90 specimens belonging to six species ranging in size from 3.7 to 21.4 mm (Table 1). Sixty-three (70%) samples yielded valid sequences in both directions that matched a portion of the Control Region of the horse (Equus caballus Linnaeus, 1758). MegaBLAST searches resulted in high-confidence matches with an average E-value of 4.62 E-68 (sd = 3.15 E-67, range: [2.49 E-66,6,4,07 E-79]) and near-complete pairwise sequence identity to accessioned, longer horse Control Region or full mitogenome sequences (mean = 98.62%, sd = 1.27%, range: [94.55,100]). The HVR1 nucleotide sequences and the ten best BLAST hits are available at http://kolokolab.org and upon request from S.-O. Kolokotronis. The recovery success of PCR products and DNA sequences differed across dung beetle species. All Onthophagus pennsylvaniae individuals yielded usable DNA sequences, Aphodiopsis ruscicola had a 90% sequence recovery rate (9 out of 10 samples), while other species were less successful, e.g.
Our main hypothesis was that body size would be a predictor of successful detection of host DNA because larger dung beetles consume larger quantities of dung. Several specimens of Geotrupes blackburnii, Onthophagus taurus, and Copris minutus in this study had visible bolus and we expected that these larger quantities of dung would have a higher likelihood of being PCR-positive. Conversely, we expected samples from smaller specimens and those in which no gut contents were visible would be less likely to contain detectable host DNA. However neither the presence of visible gut contents nor body size had any impact on the detection of host DNA. Besides variation in the mass of each meal driven by beetle body size, we expected that the total amount of mammal DNA in beetle gut contents would also be influenced by the time the beetle spent feeding before capture. Furthermore, the location of the meal may influence the amount of host DNA as beetles feeding from the periphery of the pat may be ingesting a larger amount of host epithelial cells. Our experimental methods did not allow for control of the duration of each feeding event, or the location of each event within the pats but these factors also appear to be unimportant in determining the probability of detection of host DNA.

Overall, despite its critical importance in ecosystem ecology and conservation biology, our understanding of dung beetle diets is still woefully incomplete and neither the identities of the host species nor the degree of dietary plasticity of most beetle species have been adequately characterized. This is in part a result of standard ecological methods for studying dung beetle diets in the field and the laboratory. Observations of dung beetle associations with a dung pile suggest a trophic link, but are not by themselves sufficient to demonstrate that feeding will occur (Cave 2005). Beetle-mammal associations ascertained by baiting in the field may only reflect search or exploratory behaviors rather than feeding preference (Dormont et al. 2004). In the laboratory, cafeteria experiments were beetles were starved and presented with dung of a range of species are often used to study dung beetle diets. However, observations gained through cafeteria experiments may be unreliable due to altered physiological states in the beetles, the condition of the fecal samples, and other biophysical factors. Moreover, these methods are not necessarily reflective of the composition and structure of natural trophic networks. Using molecular and genetic tools to investigate beetle diets allows researchers to bypass these limitations.

Contamination by the host’s fecal DNA originating from the dung pile or bolus is always an issue in these investigations. The beetles in this study were collected by submerging horse dung pats in water. Therefore, the beetles we sampled were completely immersed in horse dung prior to and during collection. Very low amounts of horse dung were also present in the storage medium. However, we believe that the cleanup and sterilization steps we undertook prior to the dissection, along with a careful dissection technique minimized contamination of hemocoel contents with environmental and dung pat DNA.

Future studies are needed to assess the full potential of these methods in understanding beetle-mammal networks. Field or laboratory studies using beetles feeding on a variety of mammal species would contribute to evaluating the sensitivity of these methods when beetle intestinal content may include dung from more than one mammal species. Whether beetles feeding on dung of carnivores may be PCR-positive for the carnivores’ prey species should be evaluated, as this may not accurately reflect the beetles’ dietary preferences.

In summary, our results suggest that DNA-based analyses of dung beetle gut contents can be an efficient and cost-effective method with which to study beetle-mammal networks. Understanding the nature and strength of the linkages between dung beetles and mammals underpins our capacity to identify and quantify beetle-mediated ecosystem functions and services, as well as their sensitivity to environmental alteration (Nichols et al. 2009). These methods provide a tool for unbiased assessment of species and ecosystem specific variability in dietary preference and could be applied to monitoring the composition of local mammal communities.

Acknowledgments

E. Nichols kindly shared her beetle collection and provided invaluable logistical and technical contributions. We thank R.C. Engstrand for assistance with laboratory work, and three anonymous reviewers for their comments on an earlier draft of this article.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


