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Vertebrate environmental DNA from leaf swabs

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Terrestrial vertebrates are threatened by anthropogenic activities around the world. The rapid biodiversity loss that ensues is most intense in the tropics and affects ecosystem functions, such as seed dispersal, or may facilitate pathogen transmission[1]. Monitoring vertebrate distributions is essential for understanding changes in biodiversity and ecosystems and also for adaptive management strategies. Environmental DNA (eDNA) approaches have the potential to play a key role in such efforts. Here, we explore whether eDNA swabbed from terrestrial vegetation in a tropical biodiversity hotspot is a useful tool for vertebrate biomonitoring. By swabbing leaves, we collected eDNA from 24 swabs at three locations in Kibale National Park, Uganda and used two metabarcoding systems to catalog the vertebrate taxa in the samples. We detected 52 wild vertebrate genera, including 26 avian and 24 mammalian genera; 30 of these assignments could be refined to the species level. We detected an average of 7.6 genera per swab. This approach, with its inexpensive and simple collection and DNA extraction, opens the door for inexpensive large-scale vertebrate biomonitoring.

Metabarcoding techniques have been used to detect terrestrial vertebrate eDNA in soils[2], freshwater[3] and from invertebrates that come into contact with vertebrates or their by-products as part of their lifecycle[4]. However, due to low vertebrate detection rates, none of these substrates have become a widely adopted source of eDNA for terrestrial vertebrate biomonitoring. As new substrates are explored, it was recently demonstrated that terrestrial vertebrates can be detected through collection of airborne particles[5], by swabbing vegetation for single-species detection[6] and by using rollers or sticky tape to collect eDNA from tree trunks and branches (e.g. 16 mammal species detected in 94 samples[7]; five vertebrate species detected in 14 samples[8]). This shows that vertebrates leave their DNA in the environment both as airborne particles and on the vegetation they come into contact with or when particles from air settle. In addition, the collection of vertebrate and invertebrate eDNA from rainwash[9] and flowers[10] suggests that DNA can stick to diverse plant parts. Here, we tested whether straight-forward swabbing of sub-canopy vegetation is sufficient to collect enough eDNA to describe terrestrial vertebrate communities in a diverse tropical rainforest.

We collected swabs at three locations in the interior of Kibale National Park, a biodiversity hotspot in East Africa. Taking precautions to avoid contamination, for each sample collection we dipped a swab in nucleic acid preservation (NAP) buffer and swabbed a random selection of leaves continuously for three minutes. We stored the resulting 24 swabs individually in NAP buffer at ambient temperature until arrival at the laboratory in Copenhagen, Denmark, where they were stored at –20°C prior to extraction. Laboratory space and precautions regarding contamination, metabarcoding and sequencing principally followed Lynggaard *et al.*[5]. Two primer sets targeting the mammalian 16S and the vertebrate 12S regions were employed, with five PCR replicates per extract and primer set incorporated alongside negative and positive controls. Sequencing was carried out on an Illumina MiSeq platform. Sequences were processed and amplicons taxonomically assigned using OBItools (v3.0.1b21) with stringent criteria to categorize a taxon as present. We removed detections of human and domestic animals (Supplemental information).

Despite the small sample size, we detected 52 vertebrate genera (Figure 1), including one amphibian, one ray-finned fish, 26 avian genera spanning 10 orders, 24 mammalian genera spanning five orders. All detected genera are known to occur in Kibale National Park (Table S1). The detection of the catfish (*Clarias* sp.) on terrestrial vegetation was unexpected; however, catfish are found in many of the rivers in the forest, walk on land and are consumed by avian predators. Fecal deposition of catfish DNA by birds appears a likely source, but further research is needed to assess the sources of eDNA on leaves, not just for catfish DNA but for all of the taxa detected. The previous demonstration that vertebrate DNA can be collected from air[5] suggests that airborne eDNA is widely distributed and may get deposited and accumulate on leaves. Indeed, the properties of some leaf surfaces, such as a wax coating, indentations and stickiness, might represent an ideal DNA trapping surface. While we here focused on vertebrate detection, testing for the DNA of additional taxonomic groups, such as arthropods and fungi, is an exciting future research direction. Further research is also needed to understand the persistence of DNA on leaf surfaces as well, as such data will help understand the spatial and temporal scale at which eDNA biodiversity estimates reflect an environment.

After filtering, the number of sequences assigned to vertebrates found in Kibale ranged from 8,293 to 457,460 per sample ($\bar{x}$ = 245,556, median = 271,589). The vertebrate detection rate per swab was high, with an average of 7.6 genera detected per swab (range = 3–12; Table S1). Thirty of the 52 detected genera could confidently be assigned to the species level (100% sequence identity; Table S1), including representatives from all of the mammalian orders and from nine of the ten bird orders detected. For mammals, we detected volant (3), arboreal (5) and terrestrial (5) species (Figure 1), including a vast range of body sizes; the smallest was the Stella wood mouse (*Hylomyscus stella*) at 19 grams and the largest the African elephant (*Loxodonta africana*) at 3.8 metric tons ($\bar{x}_{body\ size}$ = 221.5 kg; $median_{body\ size}$ = 3.5 kg). We detected bird species with insessorial (13), terrestrial (3), and generalist (1) primary lifestyles, with considerable variation in body mass ($\bar{x}_{body\ size}$ = 448.0 g; $median_{body\ size}$ = 41.6 g); the smallest bird detected was the variable sunbird at 6.6 grams (*Cinnyris venustus*) and

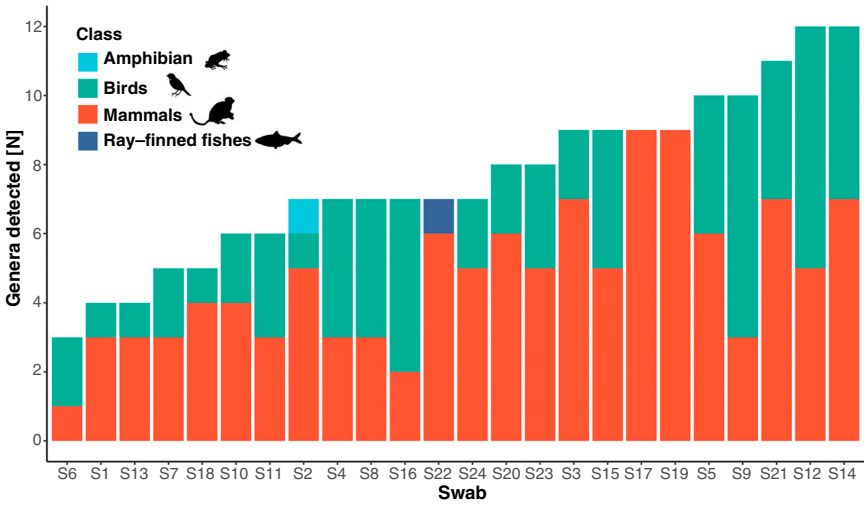

Figure 1. Vertebrate detections from eDNA leaf swabs collected in Kibale National Park, Uganda.

A stacked bar chart indicating the number of wild genera detected within each of the 24 analysed swabs. The colors indicate the number of genera detected within each class of vertebrates. Each animal image around the barchart is a representative from each order detected with the swabs, while the numbers in the lower left-hand corner of these images indicate the number of genera detected within this order.

the largest the grey crowned-crane at 3.7 kg (*Balearica regulorum*).

Survey methods based on visual observations, such as camera trapping and line transects, frequently miss cryptic, smaller, nocturnal and arboreal or flying species. Furthermore, data are time-consuming to collect and analyze. Our eDNA swabbing approach provides a tool to broadly sample terrestrial biodiversity at large scales. In contrast to collection of airborne eDNA for terrestrial vertebrate detection[5], swab collection does not require sampling devices that need thorough sterilization prior to deployment, nor power supplies and charging. While passive air sampling is also possible, it has a much lower vertebrate detection rate. Rather our approach requires only the swabs themselves, gloves, a mask, and a buffer for sample storage. While air sampling can take hours or days, swabbing takes minutes. Indeed, the 24 swabs in this study required a total of 72 minutes of sampling. Further, swabs can be collected on the spot without the need to return to a site to recover filters. Swabs have the additional advantage that DNA extraction can be easily automated with existing workflows routinely deployed in diagnostic laboratories, which could further facilitate large-scale biomonitoring efforts. Given the ease of implementation and the high diversity of wild terrestrial vertebrates detected in swabs in this study (i.e., 52 genera, $\bar{x}$ = 7.6 genera per swab), we propose that terrestrial vertebrate eDNA collected with vegetation swabs could revolutionize terrestrial biomonitoring efforts and enhance conservation efforts. The low tech and simple collection of leaf swab eDNA clearly makes it amenable to large citizen science initiatives. Ultimately, it could easily be implemented in large-scale biomonitoring efforts targeting terrestrial vertebrates and serve as a strong tool for tracking changes in ecosystem composition and function as a result of anthropogenic activities to inform adaptive management strategies.

SUPPLEMENTAL INFORMATION

Supplemental information including methods and acknowledgements can be found at https://doi.org/10.1016/j.cub.2023.06.031.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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