

Using molecular and crowd-sourcing methods to assess breeding ground diet of a migratory brood parasite of conservation concern

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Decision date: 21-Jun-2020

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/jav.02474].

Abstract

Breeding ground food availability is critical to the survival and productivity of adult birds. The common cuckoo *Cuculus canorus* is a brood-parasitic Afro-Palearctic migrant bird exhibiting long-term (breeding) population declines in many European countries. Variation in population trend between regions and habitats suggests breeding ground drivers such as adult food supply. However, cuckoo diet has not been studied in detail since before the most significant population declines in Europe began in the mid-1980s. 20th century studies of cuckoo diet largely comprised field observations likely to carry bias towards larger prey taxa. Here we demonstrate the potential value of 1) using high-throughput DNA sequencing of invertebrate prey in faeces to determine cuckoo diet with minimal bias towards large prey taxa, and 2) using crowd-sourced digital photographs from across Britain to identify lepidopteran cuckoo prey taxa during recent years post-decline (2005-2016). DNA analysis found a high frequency of Lepidoptera, including moths of family Lasiocampidae, prominent within the past literature, but also grasshoppers (Orthoptera) and flies (Diptera) that may be overlooked by field observation methodologies. The range of larval lepidopteran prey identified from photographs largely agreed with those previously documented, with potential signs of reduced diversity, and identities of key adult prey taxa were supported by molecular results. Notably, many identified cuckoo prey taxa have shown severe declines due to agricultural intensification, suggesting this has driven spatial patterns of cuckoo loss. Landscape-scale, lowland rewilding interventions provide opportunities to understand the scale of reversal of previous agricultural intensification that may be necessary to restore prey populations sufficiently to permit recolonization by cuckoos.

Keywords

Common cuckoo, *Cuculus canorus*, DNA sequencing, upland, grassland, Lepidoptera, caterpillars, Orthoptera, crowd-sourcing, conservation.

Introduction

Breeding season food is critical to adult bird survival and breeding success, and in migrant species is also a key resource for building fat reserves for migration, and entering breeding condition on arrival (Martin 1987). Following widespread declines in long-distance migrant and insectivorous birds (e.g. Vickery *et al.* 2014, Bowler *et al.* 2019), as well as in insects (e.g. Hallmann *et al.* 2017, Seibold *et al.* 2019, van Klink *et al.* 2020), knowledge of breeding ground diet of migrants is important for assessing whether reduction in insect food supply is an important driver of population changes, as has been demonstrated in resident species (e.g. Kuijper *et al.* 2009).

The common cuckoo *Cuculus canorus* (hereafter ‘cuckoo’) is a migratory brood-parasitic bird that has declined in many parts of its Palaearctic breeding range (PECBMS 2019). It preys on invertebrates that may be collected several kilometres from the habitats in which hosts are parasitised (Wyllie 1981, Dröscher 1988). Reference is often made to the species' dietary preference for (or even specialism on) large lepidopteran caterpillars, especially those with physical or chemical deterrents that are effective against other avian predators (Armitage 1978, Wyllie 1981). The bill and jaw anatomy of Cuculidae shows adaptations to handle prey at the bill base and disarm prey with toxic hairs or spines (Korzun *et al.* 2003), and cuckoos can shed their stomach lining to remove irritant caterpillar hairs (McAtee 1906, 1917). Nonetheless, cuckoos can take a range of other invertebrates (e.g. Link 1889, Wyllie 1981, Cramp 1988). A feeding specialisation or at least preference for certain Lepidoptera may help to explain the species' decline, if it reduces resilience to environmental changes that deplete prey populations (e.g. Thaxter *et al.* 2010). Significant declines in

adult abundance (Conrad *et al.* 2006, van Strien *et al.* 2019) and biomass (Macgregor *et al.* 2019) have been noted in many Lepidoptera in Europe, and species taken as larvae by cuckoos have declined more rapidly between 1975 and 2009 in the UK than moths not known to be in cuckoo diet (Denerley *et al.* 2019). Cuckoo breeding abundance shows positive trends (1995-2011) in semi-natural grass and heathland in contrast to those in agricultural or other semi-natural habitats (Massimino *et al.* 2017), and moths predated as larvae by cuckoos similarly show contrasting abundance trends (1975-2010) in these respective habitat types (Denerley *et al.* 2019).

Currently, evidence that cuckoo populations significantly rely on large caterpillars is based mainly on observational studies that are likely to be biased towards detection of such large, identifiable prey. However, molecular techniques that allow extraction, amplification and identification of prey DNA in faeces (King *et al.* 2008, Pompanon *et al.* 2012) using a region of mitochondrial gene cytochrome c oxidase I (COI) as a 'barcode' (Brown 1985) are non-invasive and offer higher and less biased taxonomic resolution.

Additionally, while it carries similar identification bias as field observation, crowd-sourced photography has demonstrated potential for combining expert identification with widespread geographical coverage, and both historical and contemporary snapshots of avian diet (RSPB 2017). Crowd-sourcing of data including photographs via social media is gaining attention as a remote-sensing tool in ecology and conservation science (Richards & Friess 2015, Di Minin *et al.* 2015, Jeawak *et al.* 2017).

Here we demonstrate 1) the use of DNA barcoding to identify cuckoo prey present in faeces collected in a refuge area of semi-natural upland grassland in

south-west England where cuckoos have overall shown a 77% decline since 1995 (Harris et al. 2019), and 2) use of crowd-sourced photography to gain a geographically broad overview of Lepidoptera taken as prey by cuckoos in the period 2005-2016, following a significant long term population decline.

Material and methods

Molecular analysis of faeces

Study site and faecal sample collection

We collected faeces from common cuckoo adults in Dartmoor National Park, UK (Fig. 1) between April and June 2017 (and one bird ringed in May 2016). 52 samples were obtained by collecting fresh faeces dropped by birds using natural perches (trees, shrubs and rocks). Seven further samples were collected through licensed mist-netting. Within Dartmoor, Warren House (50.61194°N; 3.87028°W) and Burrator (50.52333°N; 4.01528°W) were selected as valleys with scattered trees, with abundant perches and short-grazed grass beneath, facilitating detection of cuckoos and their faeces. Sites were walked from 06:00 am when weather conditions were not wet and windy. Once located, each cuckoo was watched until defecation took place. Faeces were searched for within a 3 m radius of the estimated location of defecation, and collected within 20 minutes, allowing time to watch for further defecation events or to note the bird's new location if it moved. All visibly fresh faeces found were collected in separate 8 ml vials containing 90% ethanol and refrigerated at 5°C within 12 h. For data analysis, we pooled all faecal samples collected from the same 3 x 3 m area at the same time, into groups referred to as 'sampling events'.

Mist-netting was carried out at Holne Moor, Dartmoor (50.52717°N; 3.86433°W). from 04:00 am using a triangular array of three 18 m mist-nets (30 x 30 mm mesh) (Ecotone, Sopot, Poland) with a decoy female cuckoo at the centre and a sound lure of male and female cuckoo calls, at a BTO guideline volume (Blackburn *et al.* 2006). Each trapped bird was placed for ten minutes in a veterinary carry case with a clean, laminated card floor. All faecal material deposited was collected using the same protocols as above.

DNA extraction and sequencing

DNA was extracted using a CTAB-based protocol. DNA sequencing of a 290 base-pair (bp) region of the mitochondrial COI DNA was used to confirm bird species (Hebert *et al.* 2004, Kerr *et al.* 2009) from the faecal samples, unless collected by mist-netting, together with a 157 bp region to identify prey taxa using arthropod COI primers (Zeale *et al.* 2011). Libraries for a total of 48 samples confirmed as originating from cuckoo by sequencing or collection during mist-netting, plus controls, were prepared in triplicate and 300 paired-end sequenced using the Illumina MiSeq v3 SBS reagents (Illumina Corp, USA). Details of the methods used to extract the DNA, confirm the species, and prepare, sequence and identify taxa are provided in supplementary materials.

Sequencing data analysis

DNA sequence reads from Illumina MiSeq runs were de-multiplexed, and trimmed to remove primer sequence, duplicates and low quality regions (Ewing & Green 1998, Trevelline *et al.* 2016). Dada2 (Callahan *et al.* 2016) was used to identify amplicon sequence variants (ASVs). The sequence and total number of reads of each ASV (n = 268) in each replicate PCR from faecal samples were exported into a summarising table. The mean number of unique ASVs read per

sample was 14.49 (range 3-38, supplementary materials Table A1). For taxonomic identification, ASVs were entered as search queries in the Barcode of Life Database (BOLD) version 4 (Ratnasingham & Hebert 2007) Species Level Barcode Records. We noted that Lepidoptera and other species previously given as cuckoo prey in the literature (e.g. Wyllie 1981) have accessioned sequences in BOLD. Sequences from samples were considered to match a database sequence if similarity exceeded 98% (following Clare *et al.* (2011) and King *et al.* (2015)). The species with the highest percentage similarity was taken to be the prey species of origin (following King *et al.* (2015)). 160 ASVs matched eukaryote taxa, accounting for 1,864,169 reads on the MiSeq platform. Sequences matching prokaryotic taxa were excluded. 108 read sequences of appropriate length (>150 bp) for which no match could be found through BOLD, were recorded as the aggregate result 'No Match'. UK records for each top-matching species were searched online using Google. Species with no previous records of UK occurrence were highlighted (following King *et al.* (2015)). Sequences relating to these species were assumed to originate from a UK-occurring species within an encompassing higher taxon and were included in calculations of frequency of taxa on this basis.

Diet analysis

In order to limit the influence of poorly-amplified DNA samples on detection rates of prey taxa in cuckoo diet, where possible from each faecal sample a maximum of two PCR replicates, each with library DNA concentration of >2 ng/ μ L ahead of sequencing, were included in subsequent diet analysis. Including samples for which earlier extraction or amplification were poor (probably due to natural presence of inhibitors), a total of 16 faecal samples

were excluded from diet analysis and 32 were included (2 with three PCR replicates with sufficient DNA concentration, 19 with two PCR replicates, 11 with one PCR replicate). These samples represented 27 sampling events. Two alternative treatments of replicates with more stringent, reduced sample sizes had no significant effect on final compositions and frequencies of occurrence of taxa (see supplementary materials Table A2 and A3).

We calculated frequency of occurrence of taxonomic orders, families and species as the percentage of sampling events in which a sequence matching the taxon was detected. Frequency of occurrence was also calculated for some 'functional groups' of invertebrate taxa. These were 'large Lepidoptera' (typical adult wing length > 10 mm), 'large Diptera' (typical adult wing length > 5 mm following Brooke & Davies (1989)), and 'craneflies' (dipteran families Tipulidae, Pediciidae and Limoniidae). In order to estimate the completeness of dietary diversity captured by the sampling effort, accumulation curves and asymptotic richness estimates for number of taxonomic families detected were generated in R 3.5.0 using the library VEGAN (Oksanen *et al.* 2019). In order to examine co-occurrence of taxonomic families, a correlation matrix of presence (1 or 0) of families in each sequencing event was examined and pairs of families with Pearson's correlation coefficient >0.7 were identified.

Of the 27 sampling events, 13 were from April to 11 May, while 14 were from 16 May to June. To test seasonal variation in diet, chi-squared goodness of fit tests were used to compare frequency of occurrence in these two periods for orders, families, functional groups (see above) and species of larger invertebrate for which frequency exceeded 20% across all sampling events. The two periods reflected both a natural break in the seasonal distribution of samples and

correspond to the periods before and after the estimated first cuckoo laying date at Holne Moor, where passerine nests were extensively monitored in all study years (C. R. Tyler *et al.* unpublished data). For information on macro-moth and micro-moth natural history and taxonomy we followed www.ukmoths.org.uk (2019) and Agassiz *et al.* (2013), respectively.

Crowd-sourced digital photography

The search terms “cuckoo” and “*Cuculus*” were entered in the search tools of Flickr (<http://www.flickr.com>), Birdforum Gallery (<http://www.birdforum.net/gallery/search.php>), Birdguides Gallery (<http://www.birdguides.com/gallery/>), Rare Bird Alert gallery (<http://www.rarebirdalert.co.uk/RealData/gallery.asp>) and Google Images (<http://images.google.com>). Images had to meet the following criteria: i) contained a full-grown, nutritionally independent common cuckoo handling an identifiable and wild-caught prey item and ii) identified the country and month in which the image was recorded. Plumage was used to classify each bird as juvenile or adult. Prey items in images were identified to the most specific possible taxonomic level, with all identifications made by one person (BH) with relevant taxonomic expertise (Henwood & Sterling 2020). The geographic distribution of the images extended throughout Britain, from 58 locations comprising both inland and coastal sites, though images in Scotland were more clustered to coastal and island locations and few images were available from Wales. The appearance, date, location and accompanying information were used to identify images of the same bird and prey item (referred to in subsequent text as ‘prey handling events’). Photographs of 44 prey handling

events in adult cuckoos, and 24 in juveniles, met the criteria for inclusion in analysis.

Results

Molecular analysis of faeces

Sequences (ASVs) from cuckoo faeces matched arthropods of two classes (Insecta and Arachnida) and seven orders (Lepidoptera (77.8% of sampling events), Orthoptera (59.3%), Diptera (59.3%), Coleoptera (11.1%), Hemiptera (7.4%), Plecoptera (3.7%), Sarcotiformes (18.5%)), plus one class and order each of Rotifera and Tardigrada (supplementary materials Table A4). Sequences represented 33 families across these orders (Fig. 2). From positive controls containing DNA of Lepidoptera, Diptera, Annelida, Coleoptera, Hymenoptera and Crustacea, ASVs were detected that matched species from the former three taxa but not the latter. Lepidoptera accounted for more sequence reads from the positive controls than other taxa. PCR negative controls all showed <20 reads. Rotifera, Tardigrada and Sarcotiformes are extremely small organisms and were considered to have potentially originated as contaminant DNA from the field substrate, or the bodies of prey organisms, as opposed to genuine cuckoo prey. Excluding these taxa, 27 taxonomic families were detected, and an asymptotic richness estimate suggested five further families might be detected if sample size was increased by approximately 25 faecal samples (Chao value = 31.88, standard error = 4.29) (supplementary materials Figure A1).

The order with highest frequency of occurrence was Lepidoptera. The most frequent large Lepidoptera family was Lasiocampidae (predominantly drinker

moth *Euthrix potatoria*). DNA of Nymphalidae and Limacodidae species were also detected in more than half of sampling events (Fig. 2). While the latter family is not known to occur in the study region, sequences may represent locally occurring species in the same superfamily Zygaenoidea. Occurrence of the taxa Lasiocampidae, Limacodidae (Zygaenoidea) and Nymphalidae showed pairwise Pearson's correlations of $r = 0.7$ to 1.0 ($n = 27$, $P < 0.001$). The most frequent small ('micro-moth') Lepidoptera family was Oecophoridae (Fig. 2), while families Glyphipterigidae, Tortricidae and Ypsolophidae were also detected. Oecophoridae and Limacodidae (Zygaenoidea) sequences co-occurred with Pearson's $r = 0.73$ ($n = 27$, $P < 0.001$). The orthopteran family Acrididae was the second most frequent family – specifically common green grasshopper *Omocestus viridulus*. Rhagionidae (snipeflies) was the most frequent dipteran family, and Chloropidae (grass flies) and Tipulidae (craneflies) were detected in more than 20% of sampling events (Fig. 2). DNA of cranefly-like Limoniidae and Pediciidae were also detected. All Chloropidae DNA co-occurred with that of predatory Rhagionidae (*Rhagio scolopaceus* and *R. tringarius*) which may have themselves consumed chloropid flies (Chloropidae vs Rhagionidae $n = 27$, $r = 0.73$, $P < 0.001$). Other small Diptera detected were Anthomyiidae, Bibionidae, Culicidae and Psychodidae. The small-bodied Diptera and Lepidoptera detected were considered to be potential non-prey sources of DNA in cuckoo faeces, as already suggested for the Tardigrada, Rotifera and Sarcotiformes, for example entering the body as prey of consumed taxa.

Seasonal variation in frequency of occurrence for i) orders Lepidoptera and Orthoptera, ii) 'large Lepidoptera', and iii) lepidopteran family Lasiocampidae,

(all more frequent late in the season), plus iv) families Limacodidae and Nymphalidae, v) the drinker moth *E. potatoia*, vi) order Diptera, vii) 'large Diptera', viii) the dipteran family Rhagionidae and ix) 'craneflies' (all more frequent early in the season) were all non-significant but some only marginally so ($\chi^2 < 3.71$, 1 d.f., $P > 0.05$). Frequency of occurrence of Tipulidae was significantly higher in the early season ($\chi^2 = 6.031$, 1 d.f., $P = 0.014$) (Fig. 3).

Crowd-sourced digital photography

Adult cuckoos were most frequently recorded predating *E. potatoia* and oak eggar *Lasiocampa quercus* caterpillars in all regions (England, Wales and Scotland) (Fig. 4), with one or both of these species detected in all months where images were available (April-July). These taxa accounted for 39 (88.6%) adult prey handling events. Garden tiger *Arctia caja* was captured in images from Scotland only (April-May), while the larger sample of images from England included brown-tail moth *Euproctis chrysorrhoea* (May), cinnabar *Tyria jacobaeae* (July) and unidentified Noctuidae (May). Additionally, photographs showed cuckoos predating earthworms (Lumbricidae) (April-May), and non-lepidopteran adult insects which could not be identified further. Juvenile cuckoos were most frequently recorded predating *T. jacobaeae* caterpillars (Fig. 4) (July-September), but prey also documented were adult burnet moths *Zygaena spp.* (July), and larvae of nymphalid genera *Aglais* or *Vanessa* (August), large white butterfly *Pieris brassicae* (Sept.) and fox moth *Macrothylacia rubi* (Sept.-October) (Fig. 4).

Discussion

Molecular analysis of faeces

In the first molecular analysis of faecal samples for assessing common cuckoo diet, we detected prey of a range of taxa and body sizes unlikely to be otherwise detected without close individual observation, post-mortem examination or use of invasive techniques such as stomach analysis. Our field methods could be applied to other low-population-density, perching birds of open habitats. Asymptotic richness analysis suggested that at the family level, we identified approximately 85% of the taxa taken by cuckoos in the area. The high occurrence of Lepidoptera in our analysis supports the previous field observation-based conclusion that this is a key taxon in cuckoo diet. Conversely, while Orthoptera are previously reported to be predated by cuckoos (Link 1889, Abbey 1909, Bardin & Ostapenko 2019) the high frequency found by our study is unprecedented. In a quantitative study in Japan, Orthoptera were found in only one of 82 stomachs (Ishizawa & Chiba 1966). However, Orthoptera are frequent prey of Nearctic cuckoo species during the late breeding season (Beal 1898). *O. viridulus* is the most common orthopteran on Dartmoor (Davies 1987). Nymphs emerge in April and moult to adults in June (Benton 2012) so are readily available to cuckoos throughout the breeding season. While Diptera, including families detected here such as Tipulidae, have similarly been reported as cuckoo prey (Abbey 1909, Wyllie 1981), their high frequency across samples is also unprecedented. While past quantitative studies have suggested extensive feeding on Coleoptera in some examined individuals (Collinge 1925, Dement'ev & Gladkov 1966, Ishizawa & Chiba 1966), there was a relatively low frequency of Coleoptera across samples here, but the presence-based sequencing methodology limits our ability to detect dominance of taxa within an individual's faeces or diet. Overall the molecular

analysis illustrates significant consumption by cuckoos (in their refuge upland habitat) of invertebrates other than Lepidoptera, and a more generalist diet than suggested by field observational studies. Seasonal patterns of variation suggest Diptera, most notably Tipulidae, plus *E. potato*, are more frequent prey in the early season while Orthoptera and most Lepidoptera are more frequent in the late season. Molecular approaches cannot determine whether consumed prey were adults or larvae, but the significant seasonal trend in occurrence of Tipulidae in cuckoo faeces more closely reflects availability of larvae ('leatherjackets') than adults, and cuckoos have previously been reported to feed on leatherjackets (Abbey 1909, Wyllie 1981). The early season diet has additional significance as cuckoo behaviour on arrival to breeding grounds from migration suggests this food source is used to recover from migration and enter suitable condition for breeding (Lack 1968, Wyllie 1981), and future diet studies should seek to better quantify prey selected in this critical period.

Our molecular analyses were based on samples collected from a small study area and a single breeding season, largely from unmarked individuals. So, although these results cannot be generalised without further studies, they do show that these methods are well suited for studying the diet of a low population-density, insectivorous bird. We used a single primer pair to amplify prey DNA sequences, known to amplify arthropod DNA and both Gastropoda and Annelida. As cuckoos are known to largely predate invertebrates, probability of routine failure to detect diet taxa as a result of primer pair choice was expected to be low, but use of additional primer pairs would enable additional detection of any vertebrate or plant material (Bardin & Ostapenko 2019). Given the indirect nature of detecting presence of DNA rather than

identifying prey visually, we acknowledge that some detected taxa may have originated in cuckoo faeces by non-prey routes. For example, entry as a contaminant on the surface where faeces were collected, or on, or within, prey organisms may be the route by which Sarcoptiformes, Rotifera and Tardigrada entered faecal samples. However, extremely small species within Lepidoptera and Diptera were also detected. There is limited reference to such taxa in the literature on cuckoo diet (e.g. Lepidoptera of genus *Tortrix* highlighted by Wyllie (1981)), so it is less clear whether these taxa are preyed upon. However, non-prey explanations could include prey of larger consumed taxa, or incorrect identification as a result of either high representation of a related taxon in the sequence database, or sequence similarity with other taxa. There are instances suggesting each of these routes in the present dataset; for example, full co-occurrence between tiny Chloropidae and predatory Rhagionidae, high Pearson's correlation between some Lepidopteran families, and evidence of extensive barcoding effort directed to the species-rich Oecophoridae in the BOLD database.

The impact of non-prey routes to detection on interpretation of results of molecular diet studies can be reduced by knowledge of the invertebrate community of the study area, but the collection and barcoding of an extensive range of study area invertebrates (as in King *et al.* 2015, Trevelline *et al.* 2016) was beyond the resources of this study. We used a positive control DNA mixture to assess whether DNA from different taxa varied in amplification success, and the results suggested that Lepidoptera, Diptera and Annelida DNA was more readily detected than DNA of Coleoptera, Hymenoptera or Crustacea. Overall, molecular methods are expected to benefit from sequencing and

established knowledge of species of target taxa in the study area, and parallel use of other diet study methods (or further, controlled experiments comparing methods) to help disentangle relationships between species and therefore clarify results of diet analyses.

Crowd-sourced digital photography

Our analysis of crowd-sourced photography suggested that drinker *E. potatoria* and oak eggar *L. quercus* larvae are the most frequently recorded Lepidopteran prey in Britain. These are among the largest and most identifiable caterpillars in Britain and are especially likely to be identified from photographic images. However, their prevalence in the diet is supported by frequency of Lasiocampidae and *E. potatoria* in the molecular results. Garden tiger *A. caja*, once a common and widespread but now rapidly declining species (Conrad *et al.* 2002, 2006), is equally large and identifiable but occurred at very low frequency and only in Scotland, suggesting that variation in frequency in photographs does not relate only to easily-identifiable species. Cuckoo predation of earthworms is previously documented in both adults and juveniles (Ackermann & Cable 2012, Bardin & Ostapenko 2019) and the dates of photographed predations suggest they are mainly selected by adults early in the season. The analysis indicated that the prey of juvenile cuckoos was predominantly cinnabar *T. jacobaeae* larvae, previously documented by observational studies (Crawshaw 1963, Wyllie 1981). Overall, the photographic analysis suggests that there may have been a reduction in diversity of lepidopteran species in the cuckoo diet since pre-decline observational studies, such as those summarised by Wyllie (1981), though this has to remain a cautious interpretation with this relatively small data set. Comparably, a

reduction in dietary diversity was seen in linnets *Linaria cannabina*, nestlings of which were fed a diversity of arable and grassland weed seeds in the 1960s, but whose diet had become reduced to predominantly two species (*Taraxacum officinale* agg., dandelion, and cultivated *Brassica napus*, oilseed rape) by the 1990s as a result of agricultural intensification (Moorcroft *et al.* 2006). Additionally, while crowd-sourced photography with centralised expert identification carries similar biases to those in field observation approaches, photography may also be subject to biases such as collection of images in photographically favourable conditions, given that diet changes in response to weather conditions are well known in other species (e.g. Brickle & Harper 1999).

Conservation implications of cuckoo diet

The Lepidoptera of highest frequency in cuckoo diet such as *E. pottatoria*, *L. quercus*, *M. rubi* and *T. jacobaeae* are all likely to be found in greater abundance in semi-natural habitats than in improved grassland or arable agricultural land as their non-adult life stages are vulnerable to a wide range of agricultural operations including intensive grazing, drainage, hedge cutting and agrochemical use. Orthoptera are also highly vulnerable to intensive land use practices. High fertilizer application, mowing and high-density livestock grazing are associated with reduced orthopteran diversity and abundance (Chisté *et al.* 2016), and conversely orthopteran populations are relatively abundant and diverse in more semi-natural and less intensively managed areas of agricultural landscapes (Marini *et al.* 2008, Rodríguez & Bustamante 2008, Weking *et al.* 2016). Many species of Orthoptera lay eggs into soil, including common green grasshopper *O. viridulus* recorded in this study (Benton 2012) so that cultivation

and trampling by grazing animals may have survival impacts on eggs and early instars. Rhagionidae detected in this study are reported to be robust to a wide range of habitats and conditions (Oboňa & Dvořák 2014). Tipulidae in their larval stages are a widely exploited food source for birds (Buchanan *et al.* 2006) and are herbivores of plant roots in semi-natural and agricultural grasslands. Improvement measures for agriculture can include targeting of tipulid larvae with pesticides (Blackshaw & Coll 1999). In a previous study of their habitat ecology as avian food sources, both Rhagionidae and Tipulidae showed similar relative abundances under traditional and intensive grassland management (Britschgi *et al.* 2006). Overall, the key taxa highlighted across the two methods in the study are notable for being largely associated with low intensity land management.

Conclusions

In demonstrating two novel approaches to studying cuckoo diet, we have also broadened the base of evidence that cuckoos take a range of invertebrates but most frequently large taxa vulnerable to intensive land management practices. The association of the large moths and Orthoptera identified as key prey in this study with semi-natural habitats and low management intensity, combined with the documented contraction of the common cuckoo breeding range to such habitats in the UK (Balmer *et al.* 2013, Massimino *et al.* 2017, Denerley *et al.* 2019), supports the hypothesis that intensification of lowland management in the breeding grounds (e.g. Fuller 1987) may have degraded those habitats sufficiently to exclude the common cuckoo as a potential breeding species by no longer supporting life cycles of its key prey taxa. Management and restoration of lowland landscapes that promotes prey life cycles could help

reverse declines in cuckoo, as has been indicated for another declining, insectivorous Afro-Palearctic migrant, the pied flycatcher *Ficedula hypoleuca* (Versluijs *et al.* 2019). Alongside conventional nature reserves and wildlife-friendly farming systems (Pywell *et al.* 2015), landscape-scale ‘rewilding’ interventions and other large-scale conservation interventions (e.g. Vera 2000, Tree 2017) provide opportunities to observe or test the scale of reversal of agricultural intensification that may be necessary to restore sufficient prey populations to permit recolonization by cuckoos.

Acknowledgements – Collection of faecal samples in the field was assisted by Stuart Croft, employed by RSPB. James Marks from Biosciences, University of Exeter, and Kenji Toyota at School of Biosciences, University of Birmingham, UK, provided specimens of *Drosophila melanogaster* and *Daphnia pulex*, respectively, for DNA extraction for positive controls.

Funding – LM was funded on a Natural Environment Research Council PhD CASE studentship with the Royal Society for the Protection of Birds (NERC award 1491749) and the University of Exeter. DNA sequencing was performed at the Exeter Sequencing Service and Computational core facilities at the University of Exeter. Medical Research Council Clinical Infrastructure awarded (MR/M008924/1). This project used equipment funded by the Wellcome Trust Institutional Strategic Support Fund (WT097835MF), Wellcome Trust Multi User Equipment Award (WT101650MA) and BBSRC LOLA award (BB/K003240/1).

Author contributions – CRT and JW conceived the original project and designed the studies with LM. LM conducted the primary fieldwork and collection of faecal samples. DC developed and AL finalized and carried out the DNA extraction method. LM processed extracted DNA for sequencing-based identification of

bird species of origin, from amplification of avian DNA to dispatch for external sequencing, and used the returned sequences to identify the bird species. AL quantified and prepared the libraries, from PCR to final pooling, for sequencing-based identification of prey taxa. KM led on all high-throughput sequencing of the libraries. HK led on the initial bioinformatics from high-throughput sequencing. LM conducted the diet analysis from this data. BH led on identifying the invertebrate prey items from photographs. LM, JW and CRT wrote the manuscript with input from all the other authors.

Conflicts of interest – The authors have no conflicts of interest to declare.

Permits – Capture of wild cuckoos from which faecal samples were collected was carried by Dave Scott under BTO licence C5831.

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Figure legends

Figure 1. Field faecal sampling locations in Dartmoor National Park. Inset shows location of Dartmoor within southern Britain, UK.



Figure 2. Rank-frequency curves of arthropod families of a) large body-size and b) small body-size in 27 cuckoo faecal sampling events (faecal samples grouped by fine location and time of collection) in Dartmoor study sites, UK. Families with frequency < 10% are detailed in supplementary materials Table A4 with all other identified taxa.

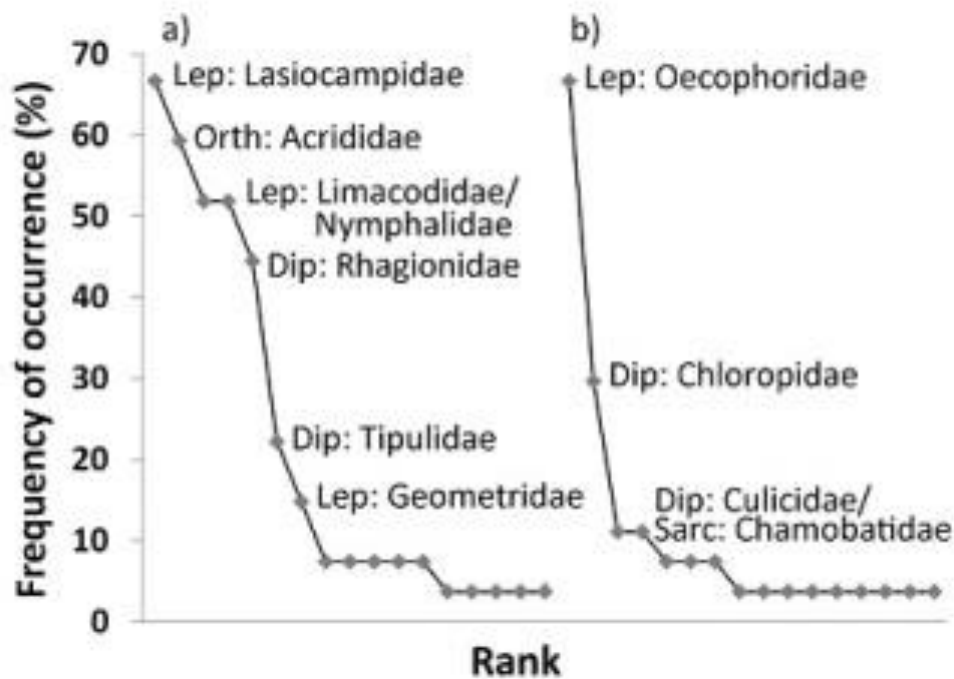


Figure 3. Frequency of occurrence of most prevalent large arthropod taxa and functional groups in faecal sampling events from cuckoos in Dartmoor, UK, for period April to May 11 (n = 13, white bars) and period May 16 to June (n = 14, grey bars). * denotes significant variation in frequency at $P < 0.05$.

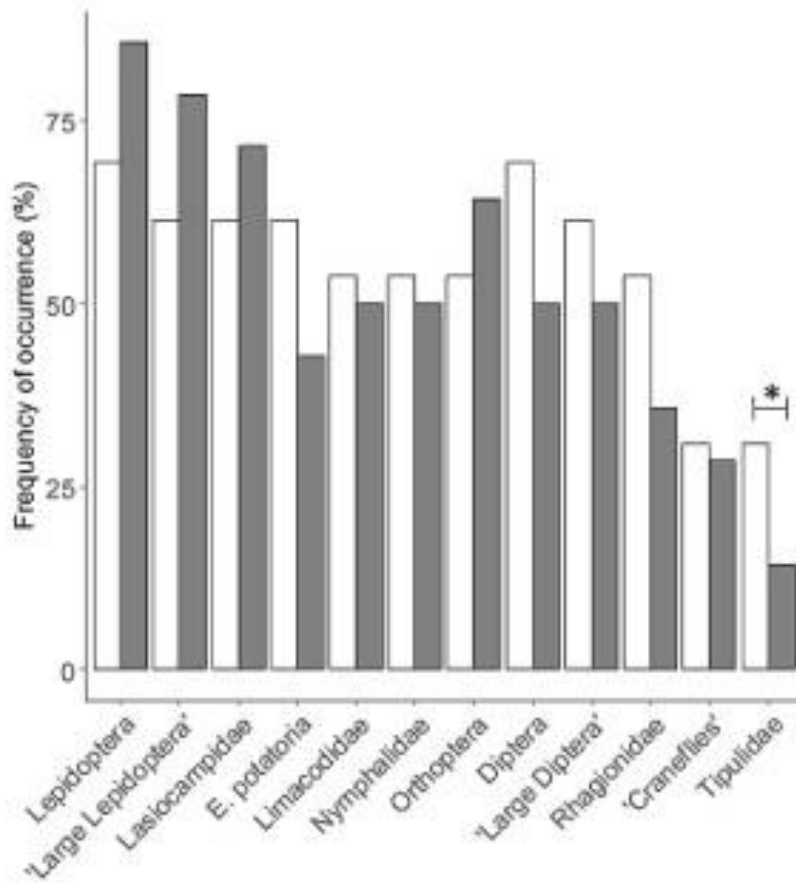


Figure 4. Counts of lepidopteran prey identified from crowd-sourced photographs of adult cuckoo prey capture events from England, Wales and Scotland, and juvenile cuckoo prey capture events from all three regions. Map dots show locations at which 1 (●) or 2+ (●) images were taken.

