Prey detection in carabid beetles (Coleoptera: Carabidae) in woodland ecosystems by PCR analysis of gut contents

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Abstract. Predatory carabid beetles are important for regulating prey abundance in terrestrial ecosystems. While surveys of carabid diet have revealed many insights into trophic interactions, the high species diversity and heterogeneous developmental stages of prey identified in the gut have made further advances difficult. In addition, the carabid gut contains partially digested and mainly soft tissue parts of the prey species, difficult to identify by traditional methods. Molecular gut content analysis (MGCA) avoids these disadvantages but to date has been limited primarily to revealing pest species in agricultural fields. Here we used MGCA to screen for the presence of Lepidoptera in carabid guts, in woodland ecosystems, in both Croatia and the UK. Data on carabids positive for Lepidoptera were compared with those from previous work on the same carabid assemblages, screened for earthworms, slugs, woodlice and springtails. In both locations, the prey group most frequently detected was earthworms, followed by slugs and Lepidoptera and then finally by woodlice and springtails. The composition of the diet changed with season, carabid sex, and carabid size. In both locations, Lepidoptera were the third most frequent prey, with 27% of carabids testing positive in Croatia and 20% in UK, suggesting that carabids could be significant predators of Lepidoptera in woodland ecosystems and may potentially play an important role in controlling moth pests.

INTRODUCTION

Carabids are important predators in soil ecosystems, where they regulate the abundance of many invertebrates including pests and invasive species (Paill, 2004; Hatteland et al., 2011; Boreau de Roince et al., 2012; Traugott et al., 2012), as well as contribute to ecosystem stability. Carabids feed on soil invertebrates, including earthworms, slugs, snails, woodlice, springtails and insects (e.g. Sunderland, 1975; Thiele, 1977; Hengeveld, 1980a; Sunderland & Sutton, 1980). They can be classified according to the composition of their diet as monophagous, oligophagous or polyphagous. The diet of polyphagous species varies between individuals, and in time and space (Hengeveld 1980a; Hatteland et al., 2013), and may correlate with prey abundance in the field (King et al., 2010), while specialists have evolved to optimise capture and exploitation of particular prey species.

Therefore, ever since the pioneering work of Forbes (1883), researchers have applied various techniques to characterize the carabid diet. At first, direct observation of carabid predation and microscopic analysis of carabid gut were used, but these approaches proved ineffective for observing predation by nocturnal species; identifying soft-bodied prey, prey partially digested before ingestion, or plant tissue; and properly classifying a high diversity of soil species with limited taxonomic data.

To overcome these difficulties, researchers have relied on techniques such as isoenzyme analysis (Paill, 2000, 2004) and both monoclonal and polyclonal antibodies (Dennison & Hodkinson, 1983; Symondson et al., 2000; McKemey

et al., 2003). More recently, molecular gut content analysis (MGCA) has largely displaced these older techniques. In MGCA, the polymerase chain reaction (PCR) is used to identify consumed prey based on molecular taxonomy. This powerful approach allows us to screen an entire community for multiple prey (Harper et al., 2005; King et al. 2010; Šerić Jelaska et al., 2014).

Predation may be determined by morphological constraints, including predator body or mandible size (Hengeveld, 1980a). Some carabid species prey on eggs and juvenile slugs in preference to adults (Paill, 2000, 2004; McKemey et al., 2001; Hatteland et al., 2010, 2011), as adults have thicker skin and secrete more mucus as a defensive behaviour (Foltan, 2004). On the other hand, King et al. (2010) found no evidence that larger anecic earthworms were avoided by *Pterostichus melanarius* in comparison to epigeic earthworm species, but that defence secretion by *Allolobophora chlorotica* may have reduced predation on this species by *P. melanarius*.

Within the family Carabidae the Carabini and Cychrini were described as specialist predators (Thiele, 1977; Hengeveld 1980a, b). As specialists, their prey composition should be the same regardless of the seasons. Studies suggest that in reality the situation may not be so straightforward.

Hengeveld (1985) found seasonal variation in prey availability to be one of the main factors responsible for dietary differences among three carabid species. Also, Paill (2000, 2004) and Hatteland et al. (2010, 2011) found differences in prey choice by carabids between seasons, depending on

slug phenology. Furthermore, carabid species, not only their prey, in temperate regions may exhibit seasonal variations in their activities (Thiele, 1977). In addition, carabids are classified into spring and autumn breeders, or larval and adult overwinterers, although time of reproduction may vary with geographical location and altitude. Niemela et al. (1989) showed some seasonal differences in activity patterns of some carabid species in Finland and in other parts of Europe.

These studies showed that carabids differ in their degree of polyphagy and specialization, and the fact that these researches rely on direct observation and non-molecular methods, as well as feeding in the laboratory, leaves open the possibility of biases and incompleteness. Using MGCA to analyze carabid diet composition may give more comprehensive details about carabid predation, which is important for understanding their usefulness as indicators of prey abundance within an ecosystem. For example, the extent to which carabids show specialized predation may affect their ability to regulate the abundance of a broad range of prey, especially certain pest species.

Moths (Lepidoptera) are among the most abundant forest insects and also some of the most significant pests of European forests, especially in deciduous forests where they predominate as defoliators (Day & Leather, 1997). Besides their importance as herbivores, Lepidoptera plays a valuable role in food webs as a prey for invertebrate and vertebrate predators including bats, birds and small mammals (e.g. Dodd et al., 2008; Heisswolf et al., 2009). The extent of carabid beetle predation of Lepidoptera is not sufficiently explored, except for caterpillar hunter *Calosoma* beetles (Weseloh, 1988). Therefore, we screened whole communities of carabids for the presence of Lepidoptera in their guts to determine which species are positive for these prey. Carabid species positive for Lepidoptera can be further tested by using species-specific primers for pests species, especially those that are major forestry pests. The most abundant species within carabid communities that are positive for Lepidoptera predation may potentially play an important role in controlling moth pests.

Here we analyzed prey DNA from the guts of predatory carabid beetle species collected in five woodland sites in order to reveal the presence of five prey groups (earthworms, slugs, woodlice, Lepidoptera and springtails). We wished to compare the diet composition among 23 carabid species and tested the hypotheses that this would vary with (i) location and species assemblage, (ii) season (spring-early summer vs. autumn), (iii) carabid sex and (iv) carabid size.

MATERIAL AND METHODS

Study sites and sampling

Carabids were collected from two woodland sites in Croatia (S1 and S2), during 2007, and three woodland sites in Wales in the UK (S3–S5), during 2010. In each country the animals were sampled over two seasons: from the end of May to the end of July, and from mid-September to the end of October.

The number of beetles collected per site is presented in Table 1. Beetles were collected by hand and by pitfall trapping using five dry traps (0.5 L plastic cups) per site over two weeks dur-

TABLE 1. Carabid species screened by PCR for the presence of five prey groups in their guts, carabid average body size (mm), sites where species were collected (1 and 2 in Croatia, 3–5 in UK), percentage of tested beetles positive for Lepidoptera and the number of prey groups detected in their gut.

Species	Species abbrev.	Average body size (mm)	Sites	N (carabids dissected)	% of beetles positive for Lepidoptera	Number of prey groups detected
Nebria brevicollis	N.bre	10	3,4,5	87	0.20	5
Abax parallelus	A.par	16	1,2	68	0.66	5
A. parallelepipedus	A.at	20	1,2,3,4,5	63	0.14	5
Carabus nemoralis	C.nem	26	1,2	18	0.17	3
C. ullrichi	C.ull	30	2	13	0.00	2
C. violaceus	C.vio	30	1,2,4,5	9	0.44	3
Pterostichus madidus	P.mad	12	3,4,5	7	0.14	4
Cychrus attenuatus	Cy.at	16	1,2	7	0.14	3
Agonum sp.	Ag.sp	8	5	6	1.00	2
C. convexus	C.con	18	2	6	0.33	2
C. intricatus	C.int	30	1,2	6	0.17	2
C. coriaceus	C.cor	36	2	6	0.50	3
Leistus fulvibarbis	L.ful	8	3,4	3	0.00	2
P. fasciatopunctatus	P.fas	15	1	3	0.00	0
B. quadrimaculatum	B.qua	4	3	2	0.00	1
Molops piceus	M.pic	6	2	2	0.00	3
P. melanarius	P.mel	12	3,4	2	0.50	2
P. transversalis	P.tra	15	1,2	2	0.50	3
Synuchus vivalis	S.viv	15	3,4	2	0.50	3
C. problematicus	C.pro	30	4	2	0.00	2
Notiophilus rufipes	N.ruf	8	2	1	0.00	2
Aptinus bombarda	A.bom	12	1	1	0.00	2
Bembidion sp.		4	4	1	0.00	0

TABLE 2. Site coordinates (WGS84 date, Simple Cilindrical projection – Google Earth).

Sites	Latitude	Longitude
S1 (CRO)	45°52′55.46″N	15°55′15.69″E
S2 (CRO)	45°52′39.26″N	15°57′55.99″E
S3 (UK)	51°31′50.63″N	3°22′23.90″W
S4 (UK)	51°34′04.45″N	3°10′47.34″W
S5 (UK)	51°34′17.05″N	3°11′44.95″W

ing the seasons mentioned above. Traps were emptied every day and beetles were placed in plastic tubes separately, transported to the laboratory and killed immediately at –80°C. They were then identified to species and sex based on morphology (Freude et al., 2004; Luff, 2007).

All sites are situated in temperate deciduous forests. UK sites were dominated with sessile oak, while at site S5 there were also ash, field maple, sycamore and sweet chestnut with an understory of dogwood, hazel and hawthorn. This was similar to the Croatian site S2 with respect to floristic composition in all layers (trees and understory), where sessile oak were dominant with sweet chestnut and common hornbeam. Site S1 was dominated by beech, and less abundant sessile oaks. Croatian sites are managed as even stands of trees of similar age/size. At the time of sampling S1 was in the mature state phase, while S2 was in transition from young to mature. UK sites S3 and S4 consisted of mature sessile oaks while S5 was in transition from young to mature stands. Croatian sites were 3.7 km apart, while the UK sites could fit into a circle with a 14 km diameter (Table 2).

Molecular analyses

DNA extraction

Beetles were collected in the field, put in the separate plastic containers and preserved at –80°C until DNA extraction. Immediately before extraction, beetles were defrosted, their foregut was removed and then used in DNA extraction (317 beetles). To test the primers for specificity, positive control DNA was extracted from the tissue of the prey species *Cydia pomonella* (Lepidoptera: Tortricidae) and from several Lepidoptera larvae from the field. Negative control DNA was extracted from 19 non-target soil invertebrate species, mainly collected from the sites surveyed in this research [Carabid beetles: *Calathus fuscipes* and *Bembidion* sp.; Other beetles: *Ocypus olens* and *Geotrupes* sp.; Hymenoptera: Ant (sp 1), Ant (sp 2), Ant (sp 3); Diptera: Crane fly larvae (sp 1); Earwigs (sp 1); Springtails (sp 1); Spiders: *Pardosa* sp. and *Erigone atra*; Woodlice: *Oniscus asellus*; Earthworms: *Eisenia foetida* and *Lumbricus castanea*; Slugs: *Deroceras reticulatum* and *Limax* sp.; Snails: *Helix aspersa*; Nematodes (sp 1)]. All extractions were performed using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. During each extraction, negative controls with no added animal tissue were included to check for potential DNA carry-over contamination during extraction.

To check for the presence of DNA after extraction and to avoid false negatives, extractions were tested by PCR using general invertebrate primers that amplify a 710-bp fragment of the mitochondrial cytochrome oxidase I (COI) gene (Folmer et al., 1994) using the same conditions as in Šerić Jelaska et al. (2014).

Screening for prey DNA in the gut

DNA extracts from the 317 carabids were screened by PCR using group-specific primers, targeting the part of mitochondrial 12S ribosomal RNA gene of Lepidoptera (LM-14259-F, TCTGCATCTTGATCTGAT; LM-14423-R, TTTGGCGGTATTTTAGTTCAT; Sutherland 2000) generating a ~165 bp amplicon.

Primers had been tested previously by Sutherland (2000) and proved to amplify 12S rRNA for 17 Lepidoptera species belonging to six families (Nymphalidae, Lycaenidae, Geometridae, Noctuidae, Oecophoridae and Tortricidae), common in deciduous forest and shrubs (Appendix 1). Most of them are defoliators, including *Operophtera brumata*, a serious pest in beech forests.

PCR reactions (10 μL) contained 1.2 μL of extracted DNA, 5 μL of Multiplex PCR Master Mix (Qiagen), 0.2 μM of each primer, 10 μg of bovine serum albumin (New England Biolabs), and *sterile* distilled *water* (Qiagen). After an initial denaturing step at 95°C for 15 min, amplification proceeded for 35 cycles at 94°C for 30 s, 56°C for 1 min 30 s, 72°C for 1 min 30 s and a final extension at 72°C for 10 min. All PCRs included a positive control (*Cydia pomonella* DNA) and a negative control (sterile water instead of DNA). PCR products were separated on a 2% agarose gel for 40 min at 120 V and visualized with 0.075 μg/mL ethidium bromide.

All samples were screened for Lepidoptera twice using the same PCR conditions. PCR reactions showing a band of the appropriate size on the agarose gel was considered positive and used in further analyses. Cross-amplification tests showed that none of the primer pairs amplified carabid DNA or the DNA of 19 non-target soil invertebrate species.

The same set of DNA gut extracts from 317 field-caught carabid beetles screened for Lepidoptera, were previously screened by PCR using different primer sets in Šerić Jelaska et al. (2014): general primers for earthworms (Harper et al., 2005), woodlice (Jarman et al., 2006), springtails (Kuusk & Agustí, 2008) and arionid slug species (*Arion hortensis*, *A. distinctus*, *A. silvaticus*, *A. subfuscus*) (Dodd, 2004; Harper et al., 2005), as well as species-specific primers for the limacid slugs *Limax cinereoniger* (Šerić Jelaska et al., 2014) and *Deroceras reticulatum* (Dodd, 2004; Harper et al., 2005). The results given here and the previous data set on prey presence in carabid guts using these additional primers were compared and used to analyse overall trophic interactions.

Statistical analyses

Statistical clustering was calculated based on Bray Curtis similarity measures and group average linkage. Cluster analyses, Pearson correlations and Chi-square tests were performed, and the corresponding figures prepared, using R (version 2.11.1, R Development Core Team, 2011), Primer 6 (PRIMER-E Ltd. 2006), and Gephi 0.8.2 beta [Common Development and Distribution License (CDDL) & GNU General Public License 2008–2012].

RESULTS

Carabid beetle assemblages

Of the 317 carabids collected, 179 were sampled in Croatia and 138 in Wales, UK; 162 were collected in spring-early summer and 155 in autumn. Approximately equal numbers of males and females were caught in spring-summer, while slightly more males (57%) were collected in autumn. The 317 carabids belonged to 23 species. The most abundant at UK sites were *Nebria brevicollis* and *Abax parallelepipedus*; the most abundant at Croatian sites were *Abax parallelus* and *A. parallelepipedus*, followed by *Carabus nemoralis* and *C. ullrichi* (Fig. 1). Of the three most abundant species in the overall sample, there were more male (44) than female (16) *A. parallelepipedus* in summer, with only four females and no males in autumn. *Nebria brevicollis*, an autumn breeder, were dominant in autumn with more males (47 individuals) than females (30) collected,
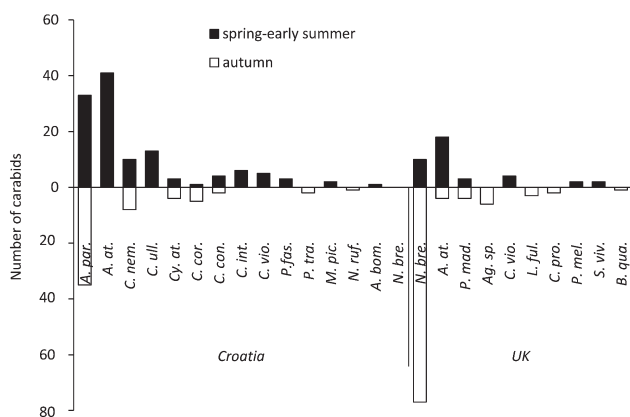
Fig. 1. Frequencies of carabid species sampled during two seasons at woodland sites in Croatia and UK. Full names and abbreviations of carabid species are listed in Table 1.
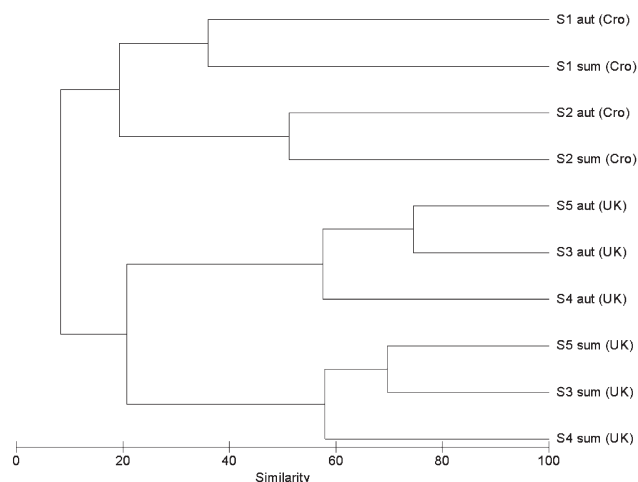

Fig. 2. Dendrogram based on Bray-Curtis resemblance showing similarities in carabid species composition and relative abundance at five study sites sampled in spring-summer and autumn.

and for *Abax parallelus* collected in both seasons, more females (23) than males (10) were collected in summer, and similar numbers of both sexes were collected in autumn (16 females and 19 males).

Cluster analysis used to identify similarities in the species composition of carabid communities showed that UK sites (S3–S5) clustered together in both seasons (Fig. 2). Cluster analyses within each season showed only 36% similarity in carabid species at Croatian site S1 and 50% similarity at Croatian site S2 (Fig. 2).

MGCA after screening carabids for Lepidoptera

Of 317 beetles screened, 76 (24%) contained Lepidoptera DNA in the gut. Of these, 19 contained only Lepidoptera, while the rest also contained other prey (earthworms, slugs, woodlice or springtails). Of the 23 carabid species identified in our field samples, 14 were positive for Lepidoptera (Table 1), with the highest percentage of positives within *A. parallelus* (66%), followed by *C. violaceus* (43%) and *C. convexus* (33%). *Nebria brevicollis* and *A. parallelepipedus* had 19.5% and 14.3% of positives, respectively.

More positives were detected in autumn (45 beetles, 29% of 155) than in spring-early summer (31 beetles, 19% of 162). Five species were sampled in both seasons at some sites as *N. brevicollis* at S3, S4 and S5, *A. parallelus* at S2, *C. convexus* at S2, *C. nemoralis* at S1 and S2 and *P. madidus* at S3. There was no significant difference in numbers testing positive per species per site in autumn vs. spring-early summer (chi-square value = 0.6327, df = 7, p-value = 0.9988).

Diet analyses including presence of other prey groups in carabid gut

MGCA showed the presence of all five prey groups in the gut of carabid assemblages sampled from UK and Croatian sites in both seasons: earthworms, slugs, woodlice, springtails and Lepidoptera (Fig. 3). Of 317 individuals, 232 (73.2%) were positive for at least one prey, among which 126 were positive for exactly one prey group, 69 for two prey groups, 34 for three prey groups and three for

four groups. A substantial proportion (85 beetles, 26.8%) of the 317 individuals was negative for all five prey groups.

Among all five prey groups, earthworms were present in beetle guts most often, followed by slugs and then by Lepidoptera (Fig. 3). Woodlice and springtails were detected to a smaller extent than these three prey groups. Of the beetles positive for earthworms, 57% also contained at least one more prey group; of beetles positive for woodlice, 66% contained other prey groups; of beetles positive for Lepidoptera, 67%; and of slug-positive beetles, 72%.

The proportions of the five prey groups found in individual beetles differed between two countries (chi-square value = 9.7505, df = 4, p-value = 0.04485; Fig. 4). The greatest difference was for woodlice that were present more often in carabids from UK (18.8%), than in those from Croatia (5.0%).

We examined whether carabid beetles testing positive for different prey groups varied with carabid sex, season and sampling site. At UK sites (S4 and S5), fewer beetles were positive for earthworms than at the three other sites, while more beetles at S4 and S5 tested positive for woodlice and Lepidoptera. The proportion of beetles containing multiple prey groups was higher in autumn than in spring-early summer. Females were more positive for earthworms than for other prey groups, and they showed strong seasonality: only 9% of females sampled in autumn did not contain any of the five prey groups examined, compared to 37% of females sampled in spring-early summer. Among males, in contrast, the corresponding difference was much smaller: 24% in the autumn compared to 33% in the spring-early summer.

As these proportions have not been adjusted for differences in digestion time after feeding trials for each prey-predator combination, they could change after corrections, but still the clear trends we presented here are expected to stay unchanged.

Carabid size and number of prey groups

We examined a possible association between average carabid body size and the number of different prey groups
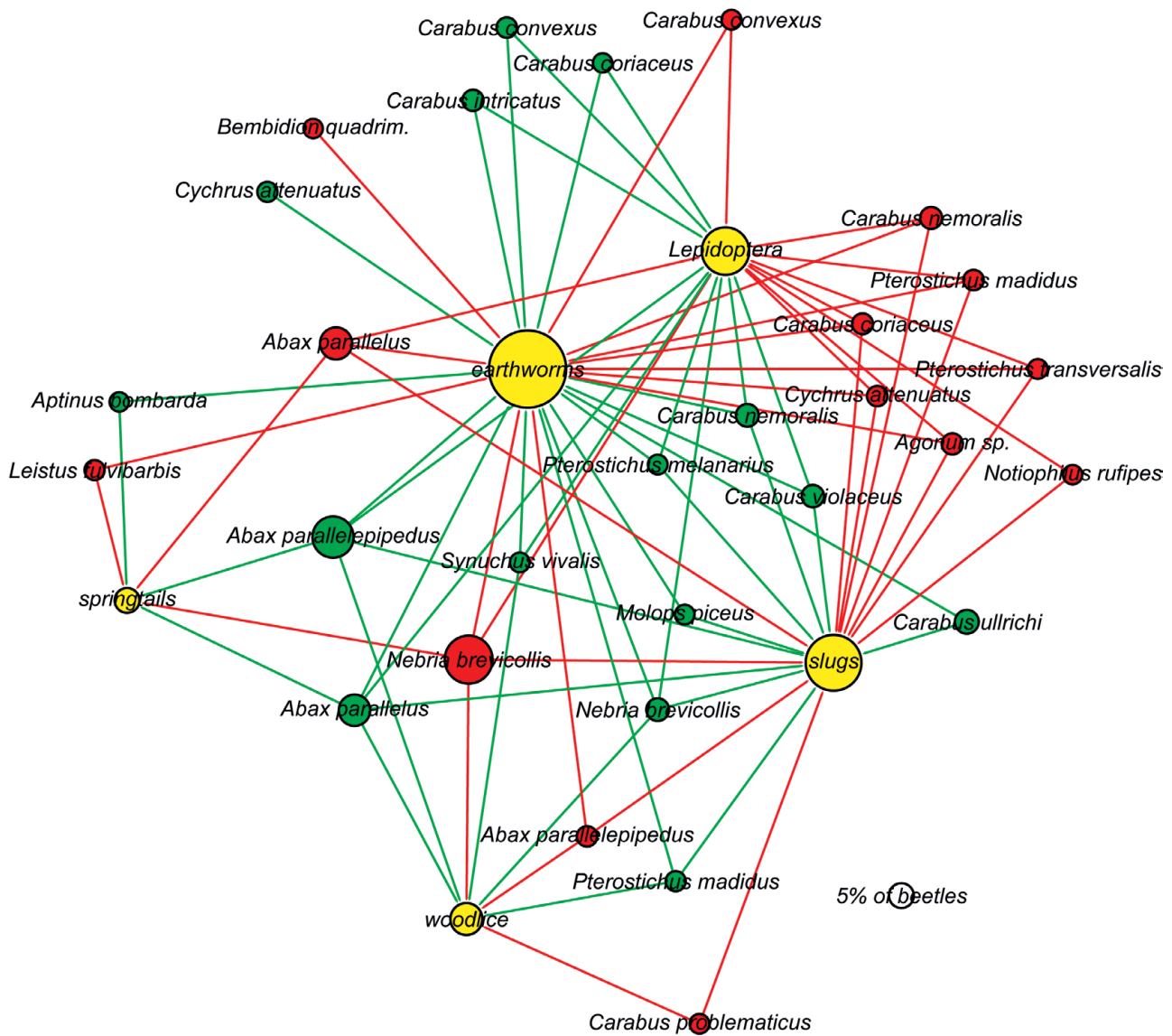
Fig. 3. Node diagrams of trophic interactions observed between carabid predator species and prey groups. The size of the nodes reflects the percentage of carabid species in the overall sample screened using MGCA. Green nodes represent beetles caught in spring-summer, red nodes beetles caught in autumn. The colour of the arrows matches the colour of the nodes and represents seasons for the given trophic interaction. Yellow nodes represent prey groups. Force Atlas model in Gephi 0.8.2 was chosen as the layout. White node represents 5% of individuals in the overall sample. Node sizes range from 40 to 160 representing 0.32 to 49% of individuals.

in their diet (Table 1). Average carabid body sizes did not correlate with the number of prey groups detected (Pearson correlation r = 0.17, N = 23). Nevertheless, there was a tendency for small carabid body size (<10 mm) to be associated with fewer prey groups (r = 0.66, N = 6).

Of the smaller beetles, *Bembidion* species were positive for earthworms, *Leistus* for earthworms and springtails, *Notiophilus* for Lepidoptera and slugs, and *Synuchus* for earthworms, Lepidoptera and woodlice. None of the large carabids (>20 mm) was found to be positive for springtails. In addition, individuals of the large *Carabus* species were not positive for woodlice, except for one *C. problematicus*.

DISCUSSION

Given the importance of carabids as predators and regulators of prey abundance in ecosystems, we used MGCA to gain more detailed insights into trophic relationships in
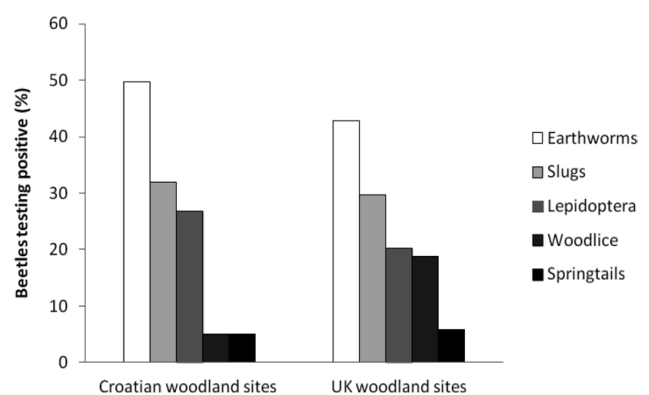

Fig. 4. Carabid predation on earthworms, slugs, Lepidoptera, woodlice, and springtails based on the numbers of beetles positive for these prey by PCR. A total of 138 individuals were analyzed from UK and 179 from Croatia.

geographically distant woodland communities. Our results show that Lepidoptera are the third most frequent prey group, after earthworms and slugs, suggesting that carabid beetles may play a role in managing pest species (like the winter month) and highlight the need for further studies to quantify those interactions. We further confirm previous findings that earthworms and slugs are the most frequent prey of carabids, especially so for *Carabus* and *Cychrus* species. Our findings confirm that prey detection in carabid guts are influenced to some extent by seasonal effects and carabid body size.

The observation that earthworms are the most important prey for woodland carabid communities is not surprising given that earthworms make up most of the invertebrate biomass in European soils (Jeffery et al., 2010). Earthworms provide nutrients that may improve carabid fitness parameters such as fecundity (Symondson et al., 2006).

Although earthworms were an abundant part of the carabid diet independently of the sampling site or season, the proportions of individuals that also preyed on slugs was significantly higher in autumn than in spring-early summer, based on eight species for which we collected individuals during both seasons (Šerić Jelaska et al., 2014). The proportions of individuals that preyed on Lepidoptera were also higher in autumn, although not significantly so. A higher proportion of beetles positive for Lepidoptera in autumn could be effected by lower ambient temperature and thus lower metabolic activities in carabids and slower DNA digestion (Von Berg et al., 2008), but also could be connected to prey density and carabid seasonal activities (i.e. King et al., 2010, Šerić Jelaska et al., 2014). In Šerić Jelaska et al. (2014) carabids were reported to consume earthworms and slugs in proportion to their field densities, whereas they consumed woodlice in lower proportions than would be expected for random predation.

Here, in this study, we didn't collect data on field abundance of Lepidoptera. Additional survey of Lepidoptera field density, including larvae and pupae on the soil, especially during population outbreak of pest species, would provide deeper insight into carabid-Lepidoptera trophic relationship and reveal the role of carabid species as predators in regulating their numbers.

Analysis of the seasonality of carabid diet, separately for males and females, showed unevenness between sexes, with less pronounced differences between the two seasons recorded for males. This could be a consequence of differences in mobility between sexes. For example, food satiated females are significantly less active than satiated males (which are actively seeking females), (Szyszko et al., 2004). Also, several field surveys confirmed that males and females differ in sex ratios over the seasons (Tyler, 2012). Differences between male and female activities could be driven by ecological factors that directly or indirectly influence beetles consumption and digestion (i.e. lower female mobility due to food satiation and thus slower digestion or higher mobility of females than males of certain species in autumn, and thus more intense foraging and more positive females for the tested prey). As we recorded similar overall number of males and females in both seasons, and more males than females of the two most abundant carabid species in autumn sample (*A. parallelus* and *N. brevicollis*), we have no evidence for higher field activity/mobility of females in autumn.

We identified eight *Carabus* and one *Cychrus* species, all of which are known to be specialist predators of earthworms, snails and slugs. Our MGCA results confirmed that earthworms, slugs and Lepidoptera were present in *Carabus nemoralis*, *C. violaceus*, *C. coriaceus* and *Cychrus attenuatus*, and earthworms were indeed the most frequent prey detected. One exception is *C. violaceus*, in which earthworms and Lepidoptera were detected with the same frequency, although only nine individuals were screened. *Nebria brevicollis* was also once considered a specialist for collembolans, but Thiele (1977) and Hengeveld (1980) excluded it as a specialist, with Thiele (1977) listing it as oligophagus. Our molecular results confirm *Nebria* to be oligophagus: it tested positive for all five prey groups examined.

Of the five prey groups that we analyzed, the only potential pests in woodland ecosystems are Lepidoptera species (such as winter moth and gypsy moth). Many *A. parallelus* individuals, for example, contained Lepidoptera in the gut, although earthworms were the most frequent non-pest component. Our findings lead us to suggest that, in addition to *Calosoma* beetles, already described as specialist predators of gypsy moth caterpillars (Weseloh, 1985), certain major earthworm predators including *Abax* and *Carabus* may help regulate some pest moth abundance. To test this possibility, future studies should examine whether the frequency of Lepidoptera species in the gut of *A. parallelus* and even in some *Carabus* species changes with the abundance of the pest moth population. At least in Croatia, this type of study should be facilitated by the fact that the population densities of most moth pest species, including winter moths and gypsy moths, are surveyed annually in national forests to prevent extensive damage to the broadleaf deciduous forest canopy (http://stetnici.sumins.hr/).

Analysis of how carabid size correlated with the types of prey detected in the gut showed that springtail prey was detected in small and medium carabids, while large carabids such as *Carabus* species predated mostly on earthworms. The gut of most large species showed only one more prey group, usually slugs or Lepidoptera. MGCA data have not been corrected for differences in digestion time as these require extensive feeding trials in time series for all predator-prey combinations. Since we recorded 23 predator species, many feeding trials would be needed, each using at least 100 predators to model all the decay curves. Thus, broad screening, as performed here, may narrow the list of potential pest regulators to the most relevant ones (those that are at high density and show a high proportion of positives). Feeding trials to model the decay rate of DNA in these predators only would be cost-efficient.

Here we have used MGCA to provide detailed insights into an invertebrate diet under field conditions and elucidate predator-prey relationships in woodland ecosystems.

We were able to screen entire woodland communities of carabid beetles to identify a range of predator-prey relationships, adding considerably to our knowledge of the trophic links involved. In particular, we provide evidence suggesting that some of the carabid species may be involved in the control of moth pest abundance, which should be analyzed in further studies focused on specific pest species with field density surveys and feeding trials to quantify interaction strengths.

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Appendix 1. Lepidoptera species amplified in PCR using primer pair Lm-14259-F and Lm-14423 in Sutherland (2000):

Nymphalidae:
  Aglais urticae
Lycaenidae:
  Quercusia quercus
Geometridae:
  Agriopis leucophaearia
  A. aurantiatia
  Epirrita dilutata
  Operophtera brumata
  O. fagata
Noctuidae:
  Amphipyra pyramidea
  Cosmia trapezina
  Eupsilia transversa
  Orthosia stabilis
  O. cruda
Oecophoridae:
  Carcina quercana
  Diurnea flagella
Tortricidae:
  Ptychloma lecheana
  Tortricodes alternella
  Tortrix viridana