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The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates

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ABSTRACT

Soils harbour an extraordinary diversity of animals which are functionally connected by their feeding interactions. Determining these feeding links, however, is difficult due to the opaque habitat the animals live in and because many prey remains found in predator gut contents cannot be identified morphologically. DNA-based gut content analysis overcomes these hurdles. This approach opens new possibilities to empirically examine below-ground feeding links by measuring prey DNA detection frequencies in various predators. Comparing prey DNA detection rates between different predator taxa, however, is not straightforward as digestion rates and thus post-feeding prey DNA detection intervals can be affected by predator identity. In the present study we examined the effect of predator identity for a range of soil-dwelling adult and larval beetles as well as lithobiid and geophilid centipedes. These predators were fed with white grubs (scarabaeid beetle larvae) and their post-feeding prey DNA detection intervals were established for small, medium and large prey DNA fragments. In geophilids the 50% detection probability for the medium prey DNA fragment was 98 h post-feeding whereas it ranged between 22 and 34 h in beetles. Among the different beetle species, the magnitude of the differences in detection intervals was strongly affected by the size of the prey DNA fragment targeted: the intervals showed the highest similarity in the medium-sized prey DNA fragment which was most frequently detected. Predator biomass and meal size rarely affected post-feeding prey DNA detection intervals. Our findings suggest that prey DNA detection success is similar within taxonomically related species of soil-dwelling macro-invertebrates such as beetles and centipedes. Between these groups prey detection can be adjusted according to taxon-specific digestion rates for better comparisons. Moreover, the magnitude of the predator identity effect is modulated by assay sensitivity: highly sensitive assays which target small- to medium-sized prey DNA fragments are recommended as these minimize the interspecific differences in post-feeding prey DNA detection intervals.

1. Introduction

Soils are characterized by an extraordinary richness in animal taxa which play an important role within the below-ground system (Fitter et al., 2005). To better understand the functional role of the soil fauna it is inevitable to examine the feeding relationships of these animals (Scheu, 2002). However, there are several hurdles which need to be overcome to unravel the trophic interactions of soil-dwelling animals under natural conditions: for example, direct observation is restricted to artificial setup (e.g., Gunn and Cherrett, 1993) or prey remains within the gut content are often not microscopically discernible as many below-ground animals exhibit a fluid feeding mode. A means to overcome these difficulties is the detection and identification of food via DNA, a field which has undergone a rapid development within the last decade (reviewed in King et al., 2008; Pompanon et al., 2012). This methodology has successfully been employed to unravel the feeding behaviour of macro-invertebrates (e.g., Juen and Traugott, 2007; Lundgren et al., 2009; Lundgren and Fergen, 2011), of mesofauna (Read et al., 2006; Heidemann et al., 2011) and of root-feeding soil animals (Staudacher et al., 2011). Recent methodological developments enabled the examination of trophic linkages among different taxa (e.g. King et al., 2010; Eitzinger and Traugott, 2011), allowing for a detailed analysis of soil food web interactions. These efforts are sustained by the constant development of new assays for both animal (e.g., King et al., 2011; Eitzinger et al., 2013) and plant food sources (e.g., Soininen et al., 2009; Wallinger et al., 2012).

Comparing food DNA detection rates between different consumer taxa, however, is not straightforward because, among other factors, predator identity can affect post-feeding detection

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intervals. For example, it was found that in a ladybird beetle the time post-feeding for a 50% probability of detecting aphid DNA was more than double of that in a lacewing predator (Chen et al., 2000). These pronounced differences can make it difficult to compare the strength of trophic linkages between different consumers when considering raw prey detection rates (Greenstone et al., 2007). Adjusting prey DNA detection rates in predator taxa differing in their post-feeding detection probabilities has thus been proposed to allow for more realistic comparisons between them (Greenstone et al., 2010; Szendrei et al., 2010; Gagnon et al., 2011; Traugott et al., 2012).

Compared to above-ground predator–prey systems, little is known on how post-feeding prey DNA detection intervals differ between soil-dwelling predators. This hampers the interpretation of molecularly derived prey detection rates in soil food webs. The current study addresses this knowledge gap by comparing prey DNA detection success among nine soil-dwelling invertebrate predator taxa covering predatory beetles as well as geophilid and lithobiid centipedes. The selection of these predator taxa was motivated by previous work in grassland soils where centipedes and predatory beetle larvae were found to be important predators of white grubs, the larvae of scarabaeid beetles (Juen and Traugott, 2007). Aside from four species in larval stage, also adult beetles from four species were included. All of them commonly occur in agricultural land and are known as generalist predators. In the current experiments, both beetles and centipedes were fed with white grubs to establish their post-feeding prey DNA detection intervals and to compare them for significant differences. As prey DNA detection success can be affected by the size of the prey DNA fragment amplified in PCR (Agusti et al., 1999; Zaidi et al., 1999; Chen et al., 2000) and the sensitivity of the primers (Sint et al., 2012), we examined the prey detection intervals for small, medium and large prey DNA fragments. Also, the biomass of the predators, the meal size and the relative biomass increase due to feeding was determined to assess how much the predators differ in these parameters and whether this affects the prey DNA detection intervals.

2. Materials and methods

2.1. Soil arthropods and feeding experiments

Within the feeding experiments soil-dwelling predatory beetles and centipedes were examined. The former included three species of adult carabid beetles (Coleoptera: Carabidae) – *Harpalus rufipes* (Degeer, 1774), *Nebria brevicollis* (Fabricius, 1792), and *Poecilus cupreus* (Linnaeus, 1758) – as well as adult histerid beetles (*Hister* spp.; Coleoptera: Histeridae). The three carabid species are widespread over Europe and usually occupy a dominant position in ground beetle communities (Luff, 2002). Histerid beetles can also be abundant when manure is applied to fertilize fields (Traugott, 2002a). Immature predatory beetles were represented by larvae of *Poecilus versicolor* (Sturm, 1824) and *P. cupreus*, the elaterid *Agrypnus murinus* (Linnaeus, 1758; Coleoptera: Elateridae) and soldier beetle larvae from the genus *Cantharis* (Coleoptera: Cantharidae). *Poecilus* larvae are known as soil-dwelling predators with *P. versicolor* and *P. cupreus* typically found in soils of grassland and arable land, respectively (Traugott, 1998; Juen and Traugott, 2007). The predatory larvae of *A. murinus* and of *Cantharis* spp. can be found in both arable and grassland soils (Traugott, 2006; Traugott et al., 2008), with the former being endogaeic and the latter being hemiedaphic. In *Poecilus* third instar larvae and in *Cantharis* fifth and sixth instars were used for the feeding experiments, while the larvae of *A. murinus* ranged between third and eighth instar. The centipedes were represented by *Geophilus* spp. (Chilopoda: Geophilidae) and *Lithobius* spp. (Chilopoda: Lithobiidae), with the latter typically found as a soil surface dweller and the former being truly soil-living (Lewis, 2007). All arthropods were hand-collected near Innsbruck, Austria in arable fields (most beetles), extracted out of soil samples (Geophilidae) or retrieved by sieving forest litter and compost (Lithobiidae). We strove to collect sufficient individuals for each predator taxon to examine at least five predators per time point post-feeding. However, due to low catches we could not achieve this number in *N. brevicollis*, *Hister* spp., *Lithobius* spp., and *A. murinus*. The larvae of the two *Poecilus* species were reared in the lab as described in Juen and Traugott (2005) as they are hard to retrieve in the field (Traugott, 1998).

All predators were kept in a temperature chamber at constant 16 °C and a light/dark cycle of 16:8 h. The *Cantharis* spp. larvae were kept at 6 °C because they are autumn- and winter-active (Traugott, 2002b). The predators were maintained on freeze-killed mealworms (*Tenebrio molitor*; Coleoptera: Tenebrionidae) until they were used in the feeding experiment. To adjust them to the same hunger level, all predators were starved one week prior to the experiment. A starvation period of only 3 days was done for the *Poecilus* larvae before the feeding experiments started as they were freshly moulted and were therefore in need of food.

The larvae of *P. cupreus* were fed with white grubs of the cockchafer *Melolontha melolontha*; all other predators were provided with the white grubs of the summer chafer *Amphimallon solstitiale* (Coleoptera: Scarabaeidae). Larvae of *P. versicolor* were fed with grubs of either *M. melolontha* or *A. solstitiale* to compare the prey DNA detection intervals between these two prey types. The grubs were collected from grassland soils near Innsbruck. Before feeding them to the predators, the head and the gut content were removed from each frozen grub. Then the grub was sliced into four pieces and one piece each was presented to a single predator. During the 2-h feeding phase each predator was kept in a transparent 20 ml plastic tube, equipped with a piece of moistened paper tissue and covered with a perforated plastic lid. The meal size was determined by recording the mass of each predator before and after feeding to the nearest 0.01 mg. Only those predators that showed an increase in initial mass were used for the experiment.

After feeding, the predators were transferred to fresh tubes and kept without food in the temperature chamber. Several individuals of each predator taxon were frozen in 1.5 ml reaction tubes at −28 °C at several time points post-feeding, ranging between 0 and 32 h for all predators. Due to having sufficient numbers of adult *P. cupreus*, additional individuals were frozen at 48 h post-feeding; this was also true for larvae of *P. versicolor* fed with *A. solstitiale* where at both 40 h and 48 h post-feeding additional individuals were taken. In *A. murinus*, *Cantharis* spp. and the centipedes two additional time points were added at 48 h and 60 h post-feeding as, based on earlier work (Traugott, 2003; Juen and Traugott, 2007), these predators were anticipated to have lower metabolic rates and thus prolonged prey DNA detection intervals. The 0-h predators were frozen immediately after determination of their biomass post-feeding. Table 1 provides an overview of the setup of the experiments.

2.2. DNA extraction

Genomic DNA was extracted from whole predators using a cetyltrimethyl ammonium bromide (CTAB)-based protocol (Juen and Traugott, 2005). One extraction-negative control was added within every batch of 30 samples to test for DNA carry-over during the extraction process. All DNA extracts were cleaned with a silica-based nucleic acid purification kit (GeneClean, Q-Biogene, Montreal, Canada) according to the manufacturer's protocol, to remove PCR inhibiting substances (Juen and Traugott, 2006).

Table 1
Predator–prey systems, time points post-feeding employed per combination, number of predators tested per time point (*N*/time point), and total number of predators tested within each predator–prey combination ($\sum$ predators).

Predator taxon	Prey species	Time points post-feeding (hours)	*N*/time point	$\sum$ Predators
Adult *Poecilus cupreus*	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32, 48	10	80
Adult *Nebria brevicollis*	*A. solstitiale*	0, 4, 8, 16, 24, 32	2–3	15
Adult *Harpalus rufipes*	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32	8–10	62
Adult *Hister* spp.	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32	2–5	28
Juvenile-adult *Geophilus* spp.	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32, 48, 60	6–10	68
Juvenile-adult *Lithobius* spp.	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32, 48, 60	1–4	22
Larval *Agrypnus murinus*	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32, 48, 60	1–2	19
Larval *Cantharis* spp.	*A. solstitiale*	0, 4, 8, 16, 24, 28, 32, 48, 60	8	72
Larval *Poecilus versicolor*	*A. solstitiale*	0, 2, 4, 8, 16, 20, 24, 28, 32, 40, 48	9–46	298
Larval *Poecilus versicolor*	*M. melolontha*	0, 2, 4, 8, 16, 24, 32	20–29	157
Larval *Poecilus cupreus*	*M. melolontha*	0, 2, 4, 8, 16, 24, 32, 34	4–20	154

2.3. Screening predators for prey DNA

Specific primers, designed from cytochrome oxidase *c* subunit I (COI) sequences of *A. solstitiale* and *M. melolontha* (Juen and Traugott, 2005, 2006), were used to test the predator samples for small, medium and large prey DNA fragments. The primer pairs targeting *A. solstitiale* included SPE-CO1-S21/SPE-CO1-A14 (127 bp), SPE-CO1-S13/SPE-CO1-A14 (463 bp), and SPE-CO1-S13/SPE-CO1-A15 (853 bp); those targeting *M. melolontha* included M-175f/M-175r (175 bp), M-387f/M-387r (387 bp), and M-175f/M-585r (891 bp). Aside from extensive specificity tests carried out earlier (Juen and Traugott, 2005, 2006), the specificity of these primers for amplifying exclusively DNA of *A. solstitiale* and *M. melolontha* was verified by testing DNA extracts from predators and from mealworms (no amplification observed). Diagnostic PCRs were performed in 10 μl reactions containing 0.375 U *Taq*-polymerase (GeneCraft, Cologne, Germany), 0.2 mM dNTPs (GeneCraft), 1× buffer (GeneCraft), 3 mM $MgCl_2$ (GeneCraft), 1 μM of each primer, 10 μg bovine serum albumin (BSA; AppliChem, Darmstadt, Germany), 1.5 μl of DNA extract and PCR-water (Qiagen, Hilden, Germany) to adjust the volume. The thermocycler program consisted of 2 min at 94 °C, 39 cycles with 15 s at 94 °C, 30 s at 67 °C and 66 °C for *A. solstitiale* and *M. melolontha*, respectively, 45 s at 72 °C and a final step of 2 min at 72 °C. Each PCR included negative controls (PCR-grade water substituting DNA extract) and positive controls (diluted DNA of *A. solstitiale* or *M. melolontha*).

Primer pairs amplifying different fragments of the prey DNA are likely to differ in their sensitivity (Sint et al., 2012). To compare the sensitivity between the primer sets employed in this study, serial dilutions of DNA extracted from *P. versicolor* larvae frozen immediately after consuming either *M. melolontha* or *A. solstitiale* were tested. These samples provide a realistic ratio of target to non-target DNA used and are thus ideally suited for this comparison. Every dilution step of these samples was tested using the PCR mentioned above. For both prey species the primers targeting the medium-sized fragment showed the highest sensitivity resulting in detection up to a dilution of 1:500 while for the primers targeting the small and large fragments amplification of prey DNA was possible to a dilution of 1:100 only.

All samples scoring negative for prey DNA were re-assayed with general invertebrate primers (Folmer et al., 1994) to test if they contained amplifiable DNA at all. Samples showing no amplifiable DNA were removed from the analyses. The PCR mix was identical to the one described above. Thermocycling started with 2 min at 94 °C, followed by 35 cycles of 20 s at 94 °C, 30 s at 48 °C and 45 s at 72 °C and a final step of 2 min at 72 °C. All PCR products were separated in 1.5% ethidium bromide-stained agarose-gels at 90 V for 30 min and visualized on a UV-transilluminator.

2.4. Statistical analysis

Means of predator biomass before feeding, meal size and relative biomass increase of the predators within the 2-h feeding period were calculated for each predator taxon. The latter two parameters were compared for significant differences among predator taxa by calculating 95% tilting confidence limits using 9999 bootstrap resamples using S-PLUS 8.0 (Insightful Corporations, Seattle, WA, USA). The correlation between meal size and body mass was tested by Spearman's rank order correlation using SPSS 18. To compare prey DNA detection success over time, LOGIT analysis was carried out (Field, 2005). The time point for a prey detection probability of 50% and the respective 95% confidence limits were determined for each prey DNA fragment size and predator taxon using R 2.15 (R Development Core Team, 2012) and the package "drc" (Ritz and Streibig, 2005). Comparisons between fragments and predator taxa are based on 95% confidence limits; non-overlapping 95% confidence limits were interpreted as being significantly different. Backward logistic regressions were calculated in SPSS 18 with the variables time, biomass increase and meal size to find the variable(s) with the highest predictive power of detection success. In *Lithobius* spp. and *A. murinus* only 1–4 individuals per time point post-feeding were available, therefore we refrained in these two taxa from calculating the statistics described above for prey DNA detection success.

3. Results

3.1. Predator biomass, meal size and biomass increase

Predator biomass differed greatly among the nine predator taxa investigated, ranging from 1.4 mg in the histerid beetles to over 150 mg in the cantharid larvae (Fig. 1, lower panel). Adult carabids, cantharid larvae, *Lithobius* spp. and larvae of *A. murinus* showed the highest biomass, whereas Geophilidae, the larvae of both *Poecilus* species and the histerid beetles where much smaller. The high variability in biomass in Lithobiidae as well as larvae of *Cantharis* sp. and *A. murinus* was due to inclusion of different developmental stages and larval instars, respectively. The meal size correlated with the biomass of the predator (Spearman's rho 0.76, $P < 0.001$), with larger predators consuming between 15 mg and 38 mg and smaller ones between 0.2 mg and 10 mg in the 2-h feeding period (Fig. 1, middle panel). The relative biomass increase ranged from 12% to 45% among the predator taxa, with both adults and larvae of *Poecilus* showing a significantly higher increase than histerid beetles as well as larval *Cantharis* and *A. murinus* (<18%). An intermediate biomass increase (20–35%) was observed in centipedes, *N. brevicollis* and *H. rufipes*.

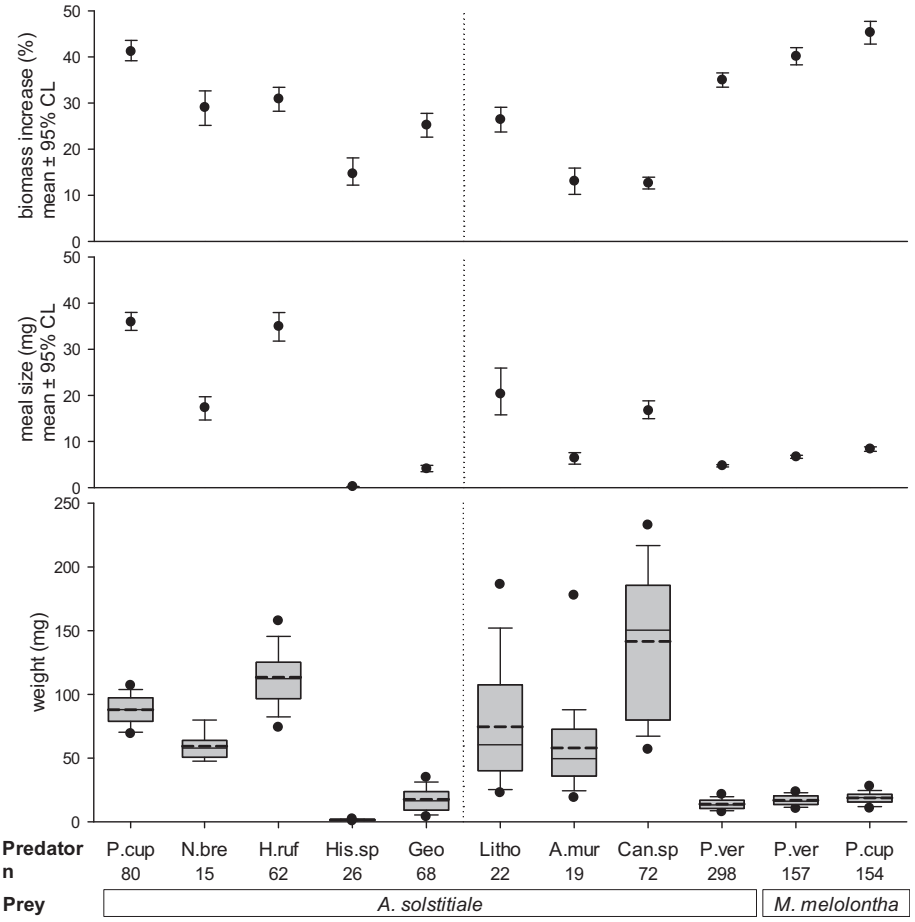

Fig. 1. Relative predator biomass increase (before/after feeding; upper panel), meal size (middle panel) and predator biomass before feeding (lower panel). For upper and middle panels mean values along with 95% tilting confidence limits are provided, the lower panel shows means (dashed lines), medians (horizontal lines), 75% (box), 90% (whiskers) and 95% (dots) percentiles for predator biomass. Adult and larval predators are displayed on the left and right of the vertical dotted line, respectively. Predator codenames are: A.mur *Agrypnus murinus*; Can.sp *Cantharis* sp.; Geo Geophilidae; H.ruf *Harpalus rufipes*; His.sp *Hister* sp.; Litho *Lithobius* spp.; N.bre *Nebria brevicollis*; P.cup *Poecilus cupreus*; P.ver *Poecilus versicolor*. The number of individuals examined per predator taxon is provided underneath the predator codename. Predators were either fed with pieces of larval *Amphimallon solstitiale* (*A. solstitiale*; nine taxa on left hand side) or *Melolontha melolontha* (*M. melolontha*; two taxa to the right).

3.2. Prey DNA detection success

Post-feeding detection intervals for small and medium prey DNA fragments were similar across beetles while they were longer in the centipede predators (Fig. 3). In beetles the 50% detection probabilities for the medium prey DNA fragment ranged between 22 and 34 h post-feeding, while they were 98 h in the geophilids (Table 2, Fig. 3). The exception to this observation was *H. rufipes* where detection success in both small and medium fragments was close to 100% across all digestion intervals tested (Fig. 2).

The effect of prey DNA fragment size was variable between the predator taxa with the large fragment showing significantly shorter post-feeding detection times compared to the small amplicons in histerid beetles, cantharid larvae and *P. versicolor* larvae fed with *A. solstitiale* (Table 2). Considering the small and medium prey fragments, no significant difference was found in prey DNA detection intervals between them except for larvae of *P. cupreus* fed with *M. melolontha* and *P. versicolor* fed with *A. solstitiale*: here prey detection intervals were significantly prolonged in the medium DNA fragment (Table 2, Fig. 3). The magnitude of the differences in prey DNA detection intervals across the beetles depended on amplicon size, with smaller interspecific differences in medium compared to small and large-sized prey DNA fragments (Fig. 3).

Adding meal size and biomass increase as variables to digestion time improved the explanatory power of the regression model only in a few cases (Table S1). When comparing the larvae of *P. versicolor* fed with either *A. solstitiale* or *M. melolontha*, only for the large prey DNA fragment a significantly longer post-feeding detection interval was found for the latter prey (Table 2). Comparing prey DNA detection success between adults and juveniles in *Poecilus*, significantly longer detection intervals were observed for small prey DNA fragments in adult beetles compared to its larval stage (Table 2).

4. Discussion

The current experiments show that post-feeding prey DNA detection intervals are affected by predator identity. However, differences which might affect comparisons of prey DNA detection rates among predators were found only between beetles and centipedes, with the latter showing on average two to three times longer detection intervals than the former. These centipede post-feeding detection intervals are similar to those of other fluid feeders such as spiders (Agustí et al., 2003; Sheppard et al., 2005; Traugott and Symondson, 2008) and heteropterans (Greenstone et al., 2007, 2010). Long prey DNA detection intervals for beetles were found in the carabid *H. rufipes* for the small and medium-sized prey DNA fragments. This might be ascribed to the omnivorous

Table 2
Estimated time points post-feeding for a 50% prey DNA detection probability for small, medium and large prey DNA fragments. Provided are the 50% prey detection probabilities in hours post-feeding and their corresponding lower and upper 95% confidence limits. Codes: A.s. *Amphimallon solstitiale*, M.m. *Melolontha melolontha*. n.c. not calculated due to most individuals testing positive at all time points post-feeding. Note that in those cases where negative detectability values were calculated an asterisk (*) is shown.

Predator–prey system	Small fragment	Medium fragment	Large fragment
	h	h	h
Adult *Poecilus cupreus* – *A.s.*	30.5 (25.4/35.5)	27.4 (23.8/30.9)	22.5 (18.4/26.6)
Adult *Nebria brevicollis* – *A.s.*	33.9 (18.5/49.2)	33.9 (18.5/49.2)	26.3 (15.6/37.0)
Adult *Harpalus rufipes* – *A.s.*	n.c.	n.c.	29.1 (21.6/36.6)
Adult *Hister* spp. – *A.s.*	31.2 (18.7/43.7)	33.8 (24.1/43.5)	11.4 (5.1/17.6)
Geophilus spp. – *A.s.*	67.4 (25.9/108.9)	97.8 (4.4/191.2)	11.6 (*/30.2)
Larval *Cantharis* spp. – *A.s.*	28.5 (19.3/37.7	22.3 (15.1/29.5)	* (*/12.4)
Larval *Poecilus versicolor* – *A.s.*	14.7 (12.7/16.8)	23.5 (20.4/26.6)	* (*/0.5)
Larval *Poecilus versicolor* – *M.m.*	19.5 (15.4/23.7)	29.6 (23.3/36.0)	21.5 (17.1/25.8)
Larval *Poecilus cupreus* – *M.m.*	12.7 (9.1/16.2)	26.4 (20.9/31.8)	11.5 (7.6/15.4)

feeding mode of *H. rufipes* which is known to consume besides animal prey a considerable fraction of plant material (Thiele, 1977). Compared to the other more carnivorous predators tested here, this species might thus be less adapted to rapidly digest animal prey, leading to a slower breakdown of smaller prey DNA fragments and the long post-feeding detection intervals observed here.

Aside from the predators' taxonomic relatedness, the magnitude of the differences in prey DNA detection intervals between the beetles was clearly affected by amplicon size: we found smaller differences in medium compared to small and large-sized prey DNA fragments. As the three prey DNA fragments were amplified by different primer pairs, not just the targeted fragment size but also the primers' sensitivities could have affected the level of prey detection. Indeed, it was the medium-sized prey DNA fragment which showed the highest detection frequency across the predators tested here. The primers targeting the medium-sized prey DNA fragment in both scarab species showed the highest sensitivity (see Materials and methods), demonstrating a primer identity effect. With regard to interspecific differences in prey DNA detection intervals, the current findings suggest that highly sensitive primers/assays targeting smaller prey DNA fragments reduce the differences in detection intervals between different consumers. On the other hand, less sensitive assays and/or those targeting larger fragments will boost taxon-specific effects in prey DNA detection success. Consequently, highly sensitive assays targeting small prey DNA fragments are recommended for comparing prey DNA detection rates between different predator taxa.

Targeting three different size classes of prey DNA fragments also allowed to examine the effect of fragment size on prey detection intervals: in the large prey DNA fragment the shortest post-feeding detection intervals were observed. However, there were several predators where among the three fragment sizes no significant differences occurred. Notably, the longest post-feeding detection intervals were most often found for medium and not, as one might expect, for the small prey DNA fragment. Although these findings contrast earlier work (e.g., Zaidi et al., 1999; Hoogendoorn and Heimpel, 2001; Agustí et al., 2003), they are in line with the outcomes of other feeding experiments (e.g., Chen et al., 2000; Traugott and Symondson, 2008). The successful amplification of prey DNA molecules ranging between 400 bp and 850 bp is also good news for the application of next generation sequencing to identify DNA from food remains (Pompanon et al., 2012): larger molecules provide more sequence information and allow for better discrimination between food items. This goes in line with an increasing sequence read length of the latest next generation sequencing platforms (Glenn, 2011).

In the Geophilidae meal size, and not time post-feeding, was the variable which explained most of the variation in prey DNA detection success for small and medium prey DNA fragments. Aside for this predator, the effects of meal size on prey DNA detection success were negligible, corroborating earlier findings (e.g., Zaidi et al., 1999; Hoogendoorn and Heimpel, 2001; Juen and Traugott, 2005). Other work, however, indicated a positive relationship between meal size and the duration of prey detection in diagnostic PCR (e.g., de León et al., 2006; King et al., 2010; Gagnon et al., 2011). Similarly, it was found that the amount of DNA from eggs of *Leptinotarsa decemlineata* consumed by *Coleomegilla maculata* by qPCR was negatively correlated with digestion time (Weber and Lundgren, 2009). The discrepancy between the current findings and the latter studies might be explained by the design of our feeding experiments where predators were fed *ad libitum* with white grubs for 2 h. The variation in both absolute meal size and relative biomass increase was comparably small within each predator taxon, therefore the effect of pronounced differences in meal size could not be explicitly tested.

Aside from time post-feeding, meal size and prey DNA fragment length, ambient temperature has been shown to affect post-feeding detection times, with lower temperatures widening them (von Berg et al., 2008). In the current study such an effect was not observed for the cantharid beetle larvae which were kept at 6 °C compared to predators maintained at 10 °C higher ambient temperatures. *Cantharis* larvae are cold-adapted (Traugott, 2003) and obviously possess digestive enzymes that are as efficient at low temperatures as those of predators living at higher temperatures. Furthermore, as the soil environment dampens ambient temperatures and buffers their fluctuations, temperature effects on post-feeding prey DNA detection intervals might be less pronounced in soil-dwelling invertebrates compared to their above-ground counterparts (but see McMillan et al., 2007).

Smaller animals show a more rapid metabolism than larger ones (Ehnes et al., 2011), which in turn should lead to shorter post-feeding prey DNA detection intervals. The present data set where the biomass of the smallest predator taxon (histerid beetles) was only 1% of that of the largest predator taxon (*Cantharis* larvae), however, do not support this hypothesis as post-feeding prey DNA detection intervals were similar across predator size classes.

Aside from the consumer, also the identity of the prey can have an effect on post-feeding prey DNA detection rates (e.g. Harwood et al., 2007; Gagnon et al., 2011; Kuusk and Ekbom, 2012; Juen et al., 2012). For *P. versicolor* larvae fed with white grubs of either *M. melolontha* or *A. solstitiale*, a significantly extended prey DNA detection interval was found for the former prey species. However, this difference was observed for the large prey DNA fragment only, indicating that, similar to predator identity effects, also the effects of prey identity are assay- and prey DNA fragment size-specific.

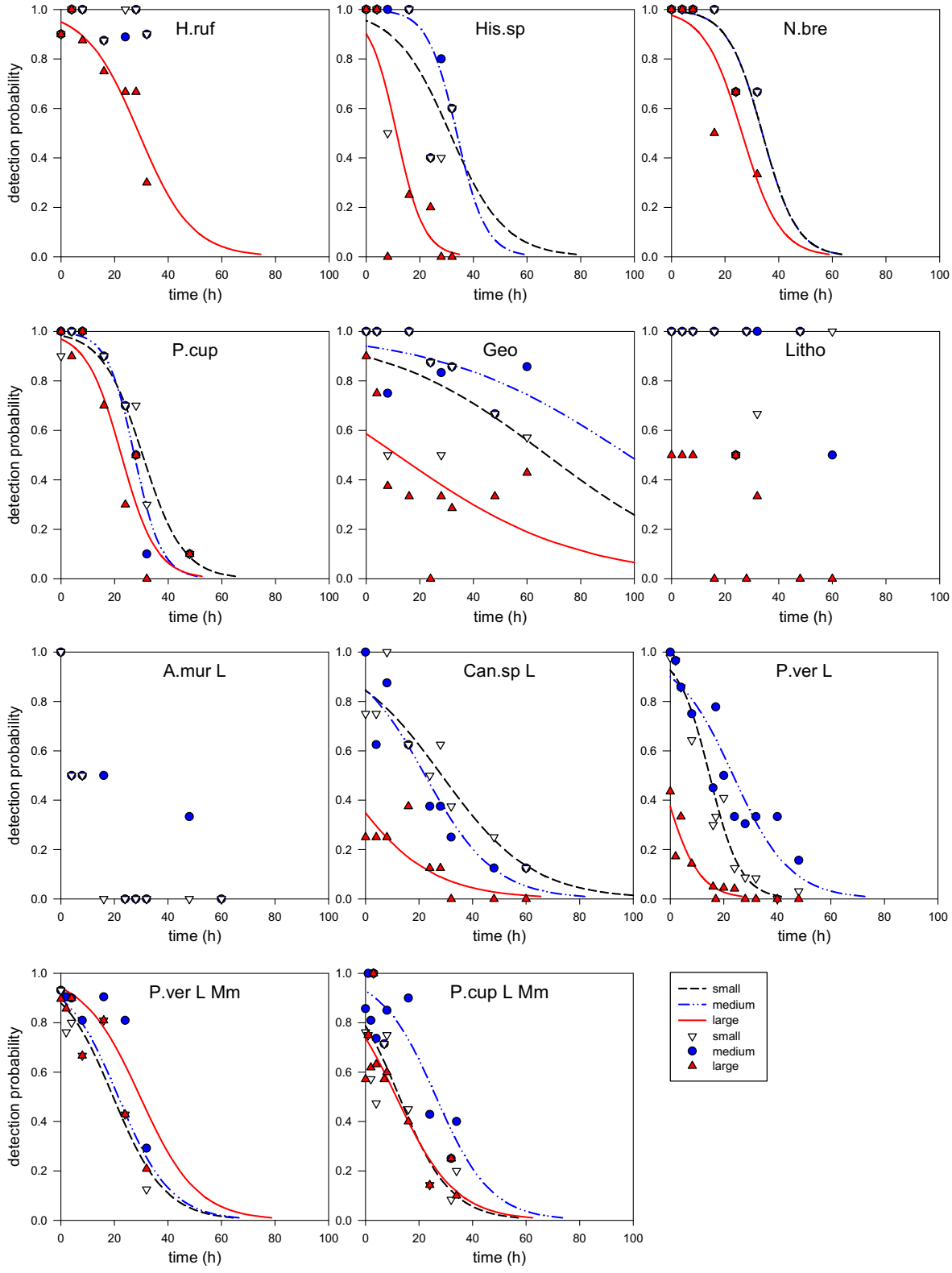

Fig. 2. Prey DNA detection success in predatory beetles and centipedes. Detection rates are provided for the different time points examined within each predator along with fitted LOGIT models. The latter are not provided for *Agrypnus murinus* and *Lithobius* spp. as only 1–4 individuals per time point were available. Prey DNA fragment sizes were 127 bp/175 bp, 463 bp/387 bp and 853 bp/891 bp for small, medium and large *M. melolontha* and *A. solstitiale* DNA fragments, respectively. Predator codenames are: A.mur Agrypnus *murinus*; Can.sp *Cantharis* spp.; Geo *Geophilus* spp.; H.ruf *Harpalus rufipes*; His.sp *Hister sp.*; Litho *Lithobius* spp.; N.bre *Nebria brevicollis*; P.cup *Poecilus cupreus*; P.ver *Poecilus versicolor*. "L" indicates larval stage and "Mm" those predators fed with white grubs of *Melolontha melolontha* instead of *Amphimallon solstitiale*. Note that in *A. murinus* all specimens were tested negative for the large fragment.

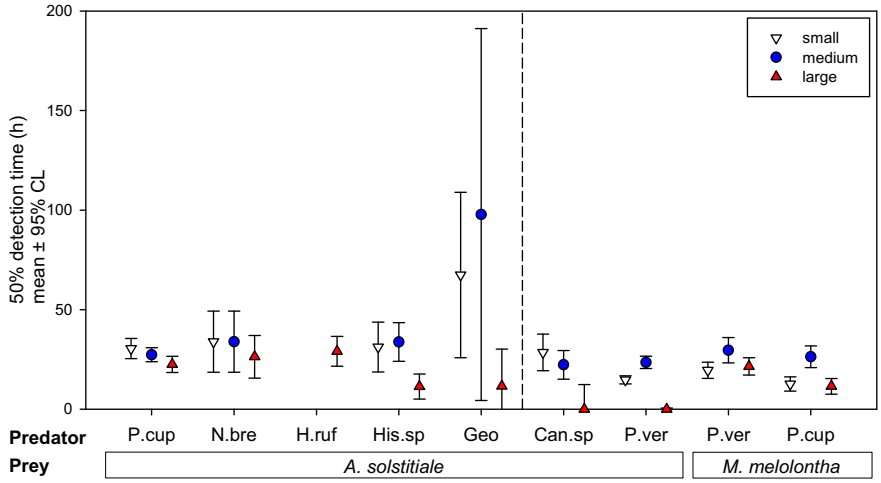

Fig. 3. Calculated hours post-feeding with a probability of a 50% prey DNA detection (±95% tilting confidence limits) for small, medium and large prey DNA fragments in soil-dwelling predator taxa. Fragments size was 127 bp/175 bp, 463 bp/387 bp and 853 bp/891 bp for small, medium and large prey DNA fragments, respectively, for predators fed with larvae of *Amphimallon solstitiale*/*Melolontha melolontha*. Dashed line separates adult (left) from larval (right) predators. Predator codenames are: Can.sp *Cantharis* spp.; Geo Geophilidae; H.ruf *Harpalus rufipes*; His.sp *Hister* sp.; N.bre *Nebria brevicollis*; P.cup *Poecilus cupreus*; P.ver *Poecilus versicolor*. Note that in *H. rufipes* the 50% detection time could not be calculated for the small and medium prey DNA fragments as the prey detection success was close to 100% at all time points tested post-feeding.

In conclusions the present data show that post-feeding prey DNA detection intervals in geophilids, with a 50% detection probability for the medium prey DNA fragment of 98 h, are on average two to three times prolonged compared to soil-dwelling larvae and adults of predatory beetles. In field studies, for direct comparisons of prey DNA detection rates between distantly related taxa such as coleopterans and centipedes the raw detection data should be adjusted to provide more meaningful comparisons (e.g. Greenstone et al., 2010; Traugott et al., 2012). However, such adjustments only make sense when the predators' feeding behaviour (e.g., feeding frequency) and the environmental conditions (e.g., ambient temperature) they live in are similar and thus will not counteract these adjustments. Moreover, the consumption of multiple prey species can affect prey DNA detection intervals (Weber and Lundgren, 2009), but this topic remains largely unexplored for invertebrates living below-ground and calls for further research. The present data also demonstrate that prey DNA fragment size and primer/assay sensitivity affected the magnitude of the interspecific differences in prey DNA detection intervals. With regard to comparing prey DNA detection rates between predator groups such as beetles, highly sensitive assays which target smaller prey DNA fragments are recommended as these minimize the interspecific differences in post-feeding prey DNA detection intervals.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.03.030.

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