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Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers

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Abstract

Collembola comprise a major source of alternative prey to linyphiid spiders in arable fields, helping to sustain and retain these predators as aphid control agents within the crop. Polymerase chain reaction primers were developed for the amplification, from spider gut samples, of DNA from three of the most abundant species of Collembola in wheat crops in Europe, namely *Isotoma anglicana*, *Lepidocyrtus cyaneus* and *Entomobrya multifasciata*. The primers amplified fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene and were designed following alignment of comparable sequences for a range of predator and prey species. Each of the primer pairs proved to be species-specific to a Collembola species, amplifying DNA fragments from 211 to 276 base pairs in length. Following consumption of a single collembolan, prey DNA was detectable in 100% of spiders after 24 h of digestion. We report the first use of DNA-based techniques to detect predation by arthropods on natural populations of prey in the field. All three species of Collembola were consumed by the spiders. By comparing the ratios of the Collembola species in the field with the numbers of spiders that gave positive results for each of those species, it was possible to demonstrate that the spiders were exercising prey choice. Overall, a single target species of Collembola was eaten by 48% of spiders while a further 16% of spiders contained DNA from two different species of Collembola. Preference was particularly evident for *I. anglicana*, the species most frequently found in spider guts yet the least numerous of the three target species in the field.

Keywords: decomposition food webs, detrital subsidy, gut-content analysis, Linyphiidae, predator–prey interactions

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Introduction

Spiders, particularly the Linyphiidae, are a major component of the generalist predator community within arable crops in Europe and elsewhere, where they feed on a wide range of major pests including aphids (Chiverton 1987; Sunderland *et al*. 1987). Spiders on arable land can attain such high densities that their webs may cover as much as 50% of the fields they inhabit (Sunderland 1999). Within wheat fields in the UK it was shown that more than 90% of species were either confined to the ground or were found both on plants and the ground (Sunderland *et al*. 1988). Thus, the majority of species spend time on the ground where they can potentially exploit not only prey such as aphids, falling from the crop above, but also epigeal invertebrates, such as Collembola, as alternative food resources.

Unlike aphid-specific parasitoids and aphidophagous predators (e.g. coccinellid beetles), spiders possess no specific adaptations that allow them to exploit aphids as prey. Indeed, laboratory studies have shown that many aphid species, though exploited by spiders, are of low nutritional

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value, or toxic, to these predators (Toft & Wise 1999). When fed on single-species diets of three common cereal aphids, *Metopolophium dirhodum* (Wlk.), *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.), egg production by adult female linyphiid spiders rapidly declined, while the aphids supported zero growth amongst juvenile spiderlings (Bilde & Toft 2001). There is therefore some doubt about the value of spiders as natural control agents for aphids, despite the fact that these predators exist in such large numbers within arable crops. Certainly many aphids fall into webs and die (Sunderland 1999) but to what extent may the spiders be acting as effective predators?

Spiders are frequently in a state of near starvation in the field (Anderson 1974; Riechert 1992; Bilde & Toft 1998) and therefore exercise little if any prey choice, eating almost anything they can capture, including aphids. One way they might survive and proliferate under such conditions is if there are adequate numbers of alternative nonaphid prey available within the crop to 'dilute' the effect of the aphid toxins or balance the amino acid requirements of the spiders (Greenstone 1979). A major source of alternative prey would be Collembola, epigeal insects that are frequently more numerous than all other insects combined on the soil beneath the crop (Harwood *et al*. 2001a, 2003). Collembola densities of up to 100 000/m^2 have been recorded in arable ecosystems (Larink 1997). Studies have shown that aphids are consumed by linyphiid spiders in the field, using polyclonal antibodies to detect aphid antigens (Chiverton 1987; Sunderland *et al*. 1987). Interestingly-Harwood *et al*. (2001b), using an aphid-specific monoclonal antibody developed by Symondson *et al*. (1999), showed that as Collembola density in the vicinity of spider web sites increased, the quantity of aphid in the guts of these spiders decreased.

Such correlations, and the fact that linyphiid spiders construct webs in areas of high Collembola abundance (Harwood *et al*. 2001a, 2003), strongly suggest that Collembola are indeed being exploited by spiders and by inference that these Collembola may be helping the spiders to receive sufficient nutrition to multiply and remain in the crop, possibly acting as control agents against aphids. Techniques that enhance detritivore densities within decomposition food webs, particularly early in the season when aphid numbers are low, may, by providing abundant alternative prey, help retain spiders and other predators in the crop and limit the expansion of aphid numbers at this critical stage. Similar processes were identified by Settle *et al*. (1996) in tropical rice, where predator numbers were enhanced by feeding on detritivores prior to the planting of the crop of rice. To determine whether Collembola could fulfil such a role, probes were required that would detect Collembola remains within spiders. As spiders ingest liquid food, particulate remains cannot be identified microscopically. The primary biochemical and molecular techniques used for such analyses involve polyclonal antisera, monoclonal antibodies and (most recently) DNA probes (Symondson 2002). Harwood (2001) characterized and tested an anti-Collembola polyclonal antiserum developed by N. E. Crook (HRI-Wellesbourne) 10 years earlier but found that it cross-reacted with many other invertebrates. Attempts to improve the specificity of the antiserum by absorption (Symondson & Liddell 1993) reduced the sensitivity of the assay unacceptably. It appeared to be the case that Collembola, perhaps because they are primitive arthropods [possibly more distantly related to insects than previously thought (Nardi *et al*. 2003)], share numerous epitopes with other arthropods. Attempts were therefore made to create more precise molecular probes using both monoclonal antibody and polymerase chain reaction (PCR)-based techniques. Here we report the results of work using the latter approach.

There has been a recent rapid development of DNA-based techniques to analyse predation by both vertebrates and invertebrates (reviewed in Symondson 2002), although until now no DNA-based approach has been used to study predation by invertebrates in the field. Studies to date have shown that the shorter the DNA sequences targeted, the longer that sequence can be detected within predators, following consumption of the prey by a predator (Agustí *et al*. 1999, 2000, 2003; Zaidi *et al*. 1999; Hoogendoorn & Heimpel 2001). They have also shown that multiple copy genes, whether nuclear (Zaidi *et al*. 1999; Hoogendoorn & Heimpel 2001) or mitochondrial (Chen *et al*. 2000; Agustí *et al*. 2003), considerably increase the probability and duration of detection. Mitochondrial genes seem to be particularly suitable for this work because much is known about levels of conservation of the different genes and parts of genes (Simon *et al*. 1994; Caterino *et al*. 2000), and they are extensively used in insect phylogenetics. There may be hundreds or thousands of copies of mitochondrial DNA (mtDNA) per insect cell (Hoy 1994), providing a large target for DNA primers, an important consideration when working with degraded DNA.

To date, DNA-based techniques have been developed both to detect parasitoids within their hosts (Amornsak *et al*. 1998; Greenstone & Edwards 1998; Zhu & Greenstone 1999; Zhu *et al*. 2000) and to detect predation on pests including mosquitoes (Diptera: Culicidae) (Zaidi *et al*. 1999), moths (Lepidoptera: Noctuidae and Crambidae) (Agustí *et al*. 1999; Hoogendoorn & Heimpel 2001), whiteflies (Homoptera: Aleyrodidae) (Agustí *et al*. 2000), aphids (Homoptera: Aphididae) (Chen *et al*. 2000), psyllids (Homoptera: Psyllidae) (Agustí *et al*. 2003) and molluscs (Dodd *et al*., 2003). Here we report, for the first time, the development of primers for the detection of a non-pest prey (Collembola) that may help to sustain an important group of generalist predators within arable fields. The only

comparable study is that by Symondson *et al*. (2000), who developed and applied monoclonal antibodies to investigate the role of earthworms in the diet of another major predator of aphids and other pests in agricultural fields, the carabid beetle *Pterostichus melanarius* (Illiger).

We undertook a field experiment to test the hypothesis that spiders were not showing preference for particular species of Collembola, but were eating them at random, in the ratios at which the different species were present in the soil. If this hypothesis proved to be incorrect then this would suggest prey choice. Direct information on the nature of such choices would indicate the species that may help to sustain spiders that are predators of aphids when aphid numbers are low. This in turn may lead to novel pest control strategies that involve enhancing the densities of those particular Collembola species.

Materials and methods

Arthropods

Three of the commonest species of Collembola at our field site at Horticulture Research International, Wellesbourne, UK (and hence those most likely to affect the spatial and temporal dynamics of the spiders), namely *Isotoma anglicana* (Lubbock) (Isotomidae), *Lepidocyrtus cyaneus* Tullberg and *Entomobrya multifasciata* (Tullberg) (Entomobrydae), were chosen for the development of prey-specific primers. Two further species of Collembola that were found in significant numbers, *Isotomurus palustris* (Muller) (Isotomidae) and *Sminthurinus aureus* (Lubbock) (Sminthurinidae), were used to test the specificity of these primers. Relevant species of spider predators, identified during earlier work (Harwood *et al*. 2001a, 2003), were collected and used to provide comparative DNA sequences to assist with primer design (see below), namely *Erigone atra* (Blackwall), *Erigone dentipalpis* (Wider) and *Tenuiphantes* (previously *Lepthyphantes*) *tenuis* (Blackwall) (Araneae: Linyphiidae), as were two species of cereal aphid, *Sitobion avenae* and *Metapolophium dirhodum* (Walker) (Homoptera: Aphididae). Further species of spider (*Oedothorax* spp.) were used during primer specificity testing, as was a model dipteran, *Drosophila melanogaster* L., obtained from a laboratory culture.

DNA extraction and sequencing

Individual arthropods (Collembola, aphids, Diptera, starved spiders and spiders from the field experiment) were homogenized in clean microcentrifuge tubes and total DNA was extracted following the protocol described by Livak (1984), with some modifications. One hundred microlitres of extraction buffer was added to the sample and incubated at 65 °C. After 30 min, 14 μL 8 M potassium acetate was added and samples were maintained on ice for another 30 min. After centrifugation at 9750 ***g*** for 15 min, 200 μL 100% ethanol was added to the pellet. The same step was repeated and 100 μL 70% ethanol was added to the pellet. A DNeasy Tissue Kit (Quiagen) was used to extract DNA from the spiders from the field experiment, as well as positive and negative controls. Pellets were all resuspended in 100 mL (200 mL for all the spiders) of TE (10 mM Tris–HCl pH 8.0, 1 mM ethylenediaminetetraacetic acid). Samples were stored at –20 °C.

The primers C1-J-1718, 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′, and C1-N-2191, 5′-CCCGGTAAAATTAAAATATAAACTTC-3′ (Simon *et al*. 1994), were used to amplify part of the mitochondrial cytochrome oxidase subunit I (COI) gene of the three target species of Collembola, the two species of cereal aphid, the Diptera and the three species of spider listed above. Amplifications were performed in 25 μL reaction volumes containing 4 μL of resuspended DNA, 0.2 mM dNTPs (Gibco), 2 mM $MgCl_2$, 0.5 μM each primer and 0.6 U *Taq* DNA polymerase (Promega) in 10× manufacturer's buffer. Samples were amplified in a GeneAmp PCR System 9700 thermal cycler for 35 cycles at 94 °C for 1 min 10 s, 58 °C for 1 min 10 s, and 72 °C for 1 min 30 s. A first cycle of denaturation was carried out at 94 °C for 2 min, and a last cycle of extension was performed at 72 °C for 5 min. Double-stranded PCR product was purified (QIAquick PCR Purification Kit; Qiagen) and sequenced (ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit; Perkin Elmer) in an ABI PRISM 3100 Genetic Analyser according to the dideoxy-chain-termination method.

Primer design and molecular tests

DNA from a minimum of two individuals of each species was sequenced in both forward and reverse directions. Sequences were aligned using SEQUENCHER Version 3.1 (575 Science Drive, Madison, Wisconsin, USA) and were corrected manually. Several pairs of primers were designed for each of the three species of Collembola in regions that were as unique as possible for each species. Guidelines proposed for the design of efficient and specific primers by Innis & Gelfand (1990) and Saiki (1990) were followed. Primers were 23–25 internal bases long, avoiding the sequences for the original universal primer. Only one pair for each species was finally selected and optimized. Primers were synthesized by Operon Technologies. Amplification conditions using these primers (25 μL volume) contained 4 μL resuspended DNA, 50 μM dNTPs (0.5 μL), 0.4 μM each primer (0.5 μL 10 μM), 3 mM $MgCl_2$ (1 μL) and 0.6 U *Taq* DNA polymerase in 10× manufacturer's buffer. Samples were amplified using the same PCR model and conditions (see above). PCR products were separated by electrophoresis in 1.5% agarose gels and were subsequently stained with ethidium bromide.

Species specificity and detection periods

The specificity of the primer pairs was tested by attempting to amplify DNA from numerous individuals of the five Collembola species plus all of the other arthropods listed above.

An experiment was then designed to ensure that prey DNA could be detected within predators that had consumed Collembola. Female spiders, *Erigone atra*, were individually confined in small (5 cm diameter) plastic Petri dishes with a charcoal and plaster of Paris base to ensure high humidity. After 2 weeks of starvation at room temperature they were allowed to consume live *Isotoma anglicana* over a period of 90 min in a controlled environment room (16–17 °C, 16 h light : 8 h dark). Once spiders had been observed to eat a single *I. anglicana* any remaining Collembola were removed and they were either immediately frozen ($t = 0$) or maintained for 4, 16, or 24 h then frozen at –20 °C for subsequent molecular assay. Eight individuals were assayed for each time period together with positive (*I. anglicana*) and negative (starved *E. atra*) control samples. Each sample was tested three times. The predators that did not feed on Collembola were discarded.

Field samples

Spiders were collected from within an on-going experiment into the effects of composting on invertebrate diversity at Wellesbourne. The hypothesis being tested was that such compost should increase the density of detritivores, potentially allowing these prey species to attract and retain high densities of generalist predators early in the season, before immigrating pests have time to become established (Settle *et al*. 1996). Full details of the experimental design and the results of this work will be published elsewhere when completed. In order, at this stage, to test the ability of the three sets of primers to detect predation in the field, spiders were collected at random within an area of winter wheat that had been treated with compost. Spiders were transferred immediately, as they were collected, to Eppendorf tubes on ice, and then stored at –80 °C prior to DNA extraction and PCR using methods and conditions described above. The underlying soil was a sandy loam, while the compost consisted of a 3-cm layer of 'Formula 3' spent mushroom compost (Noble *et al*. 1998).

Collembola density and species were recorded using four plastic field containers $38 \times 15 \times 10.5$ cm deep, filled with spent mushroom compost and sunk into the plot near to its centre. The containers were installed on 15 April at the same time as compost was applied to the rest of the plot, with the tops of the containers (and the compost within them) flush with the soil surface. By 21 June invertebrate communities within the containers (henceforth referred to as mesocosms) were considered to be representative of those in the rest of the plot. The mesocosms were then simply lifted and enclosed in a plastic bag within seconds, avoiding the inevitable disturbance and loss of rapidly moving Collembola that would result from any other form of sampling of the compost. The Collembola (and other invertebrates) were collected in two ways, by hand-searching the contents of the mesocosms (to give a measure of abundance of the larger fauna, including surface-active Collembola which would be accessible to spiders) and by processing 20% of the contents of each mesocosm (after hand-searching) using a Murphy extractor, to measure abundance of very small animals hiding deep within fragments of compost.

The numbers of different Collembola species found also represented the ratios in which the species were present in the plot, and these were first compared between sampling methods (hand-search vs. extractor sampling) using a repeated measures *t*-test. Randomization testing was used to obtain the significance level, as this may be more appropriate when the sample size is small (Manly 1997). The ratios recorded, by hand-searching of the mesocosms, of the three Collembola species for which we had developed primers, were then compared with the ratios of predator gut samples that tested positive for each Collembola species. The null hypothesis was that these ratios would not be significantly different and that therefore no prey choice was taking place. To test this hypothesis, we adopted a Monte Carlo approach to estimate the probability that the observed pattern of prey detection in gut samples using the primer sets would occur in the absence of prey choice by the spiders. The basis of the test was the 32 spiders that tested positive for Collembola in the field (24 spiders positive for one Collembola species and a further eight positive for two species, see Results). To simulate foraging by the 32 spiders, primer-positive results were allocated to the spiders at random from a hypothetical population in which the three Collembola species occurred in an identical ratio to that recorded by hand-searching of the compost (see Results). This process was repeated 5000 times to examine the range of detection patterns, using the three primer pairs, that could have been expected if no prey choice was being made. The frequency with which the observed pattern of detections (rank order for the three Collembola species) was observed over the 5000 randomizations provided a direct estimate of the probability that it could have arisen under conditions of nonselective foraging. This value is equivalent to the '*P*-value' obtained using a conventional statistical test (Manly 1997).

Results

Collembola COI markers

Fragments of 416–526 base pairs (bp) were successfully amplified and sequenced from the COI gene of *Isotoma*

Table 1 Species-specific primers designed from the COI mtDNA of *Isotoma anglicana*, *Lepidocyrtus cyaneus* and *Entomobrya multifasciata* (5′ to 3′)

Species	Primer	Sequence
I. anglicana	Ia1F	CTCTTCTATTGGCCGGAGGACTTG
	Ia4R	GCACAGGAAGTGATAGTAAAAGTAA
L. cyaneus	Lc2F	CCCACTAGCTGCTGGAATCGCCC
	Lc4R	GCACTGGGAGGGATAGTAGTAATAA
E. multifasciata	Em1F	CCCTCCTTCTTACAGGAGGTTTAG
	Em3R	TGATCTCAAGATATTCCAGGGGT

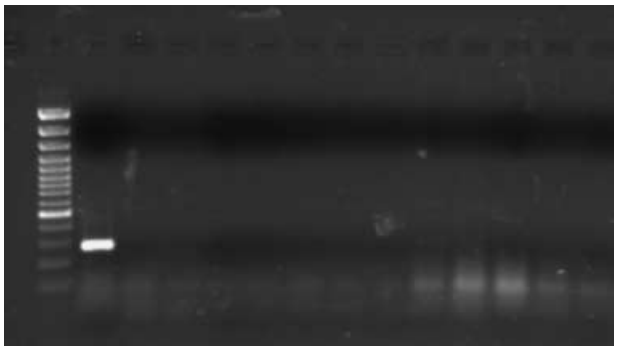

Fig. 1 Agarose gel eletrophoresis of PCR-amplified DNA using the *Isotoma anglicana*-specific COI primers Ia1F and Ia3R (276 bp). Lane 1, 100 bp molecular-size marker; lane 2, *I. anglicana*; lane 3, *Lepidocyrtus cyaneus*; lane 4, *Entomobrya multifasciata*; lane 5, *Isotomurus palustris*; lane 6, *Sminthurinus aureus*; lane 7, *Sitobion avenae*; lane 8, *Metopolophium dirhodum*; lane 9, *Drosophila melanogaster*; lane 10, *Erigone atra*; lane 11, *Erigone dentipalpis*; lane 12, *Tenuiphantes tenuis*; and lane 13, *Oedothorax* spp.

anglicana (AY383534), *Lepidocyrtus cyaneus* (AY383535), *Entomobrya multifasciata* (AY383536), *Erigone atra* (AY383537), *Erigone dentipalpis* (AY383538), *Tenuiphantes tenuis* (AY383539), *Sitobion avenae* (AY383540), *Metapolophium dirhodum* (AY383541) and *Drosophila melanogaster* (AY383542), using the general insect primers C1-J-1718 and C1-N-2191 (the numbers in parentheses are the respective GenBank accession numbers). The sequences for these prey and predator species were aligned and compared, allowing the design of four primers for each of the three target Collembola species, with four possible primer combinations for each species. After preliminary tests, the optimum primer pair was selected for each species based upon reliability of amplification. The primer pairs were Ia1F/Ia4R, Lc2F/Lc4R and Em1F/Em3R (Table 1), which amplified fragments of 276, 216 and 211 bp, respectively. Fragments between ~100 and 300 bp have been shown in previous work to be detectable in arthropod predator guts for extended periods (e.g. Agustí *et al*. 1999; 2000, 2003; Zaidi *et al*. 1999; Hoogendoorn & Heimpel 2001).

Species specificity and detection periods

When all three pairs of primers were tested for species specificity against DNA from five Collembola species, two aphids, one dipteran and four spiders, the amplification band was only present in each of the three target Collembola species. As an example, amplification patterns using the primers Ia1F/Ia4R are shown in Fig. 1.

Female spiders, *E. atra*, that had eaten a single collembolan, *I. anglicana*, were assayed for the presence of *I. anglicana* DNA in their gut using the primer pair Ia1F/Ia4R. Prey DNA was detected in all predators up to, and including, those that had digested their prey for 24 h (i.e. 100% detection).

Field results

We wished to test the hypothesis that the spiders were feeding on Collembola at random, regardless of species. Table 2 shows the species and sexes of the 50 spiders captured and the numbers of each that tested positive for one or more Collembola species in the molecular analyses. The majority of spiders (64%) tested positive for at least one species of Collembola. In total, 48% had eaten one Collembola species and 16% contained the remains of two different species of Collembola prey. Amongst the eight spiders (five *Erigone* spp. and three *B. gracilis*, all females) that contained the remains of two species of Collembola simultaneously in their guts, all three prey combinations were represented two or three times. Table 3 shows the pooled numbers (and hence ratios within the plot) of Collembola of each species collected by hand-searching of the four mesocosms and from the subsamples subjected to the Murphy extractor. A comparison of the ratios collected by the two sampling techniques found no significant difference ($P > 0.05$). This suggests that either method could be used in future trials, although the limited sample size means that further work may be needed to confirm this finding. The ratios of the three target Collembola species in the plots (mesocosm data for hand-searching, Table 3 column 2) and within the guts of the spiders (Table 3 column 4) were compared using the Monte Carlo simulation. The results strongly suggested that the spiders were not capturing the different Collembola species at random, with a low probability of the order found within the guts of the spiders arising by chance (*I. anglicana* > *L. cyaneus* > *E. multifasciata*, $P < 0.0002$). The large differences in abundance between the three Collembola species in the field plots meant that the probability of detecting the Collembola in the spiders using the three primer sets in any order other than that of Collembolan abundance was slight ($P = 0.007$).

Table 2 Species of spiders captured in the field and the numbers within which DNA from three Collembola species were detected (percentages in parentheses)

Spider species and sex	*N*	*Isotoma anglicana*	*Lepidocyrtus cyaneus*	*Entomobrya multifasciata*
Tenuiphantes tenuis (male)	8	3	0	0
T. tenuis (female)	12	1	2	0
T. tenuis total	20	4 (20)	2 (10)	0 (0)
Erigone atra (male)	3	2	0	1
E. atra (female)	1	0	1	1
E. atra total	4	2 (50)	1 (25)	2 (50)
Erigone dentipalpis (male)	2	1	0	1
E. dentipalpis (female)	6	3	3	2
E. dentipalpis total	8	4 (50)	3 (37.5)	3 (37.5)
Erigoninae sp. (male)	1	1	0	0
Erigoninae sp. (female)	4	3	1	2
Bathyphantes gracilis (male)	1	0	0	0
B. gracilis (female)	11	5	4	2
B. gracilis total	12	5 (41.7)	4 (33.3)	2 (16.7)
Oedothorax sp. (female)	1	0	0	1
All spiders	50	19 (38)	11 (22)	10 (20)
All female spider	35	12 (34.1)	11 (31.4)	8 (22.9)
All male spiders	15	7 (46.7)	0 (0)	2 (13.3)

Table 3 Numbers of each Collembola species captured by hand-searching of the mesocosms or extracted from the substrate, plus percentages of spiders that tested positive for each Collembola species (underlined) for which primers were developed

Species	Mesocosm hand-search	Mesocosm extract	Spiders % positive
I. anglicana	1	2	38
I. palustris	2	1	—
L. cyaneus	33	31	22
E. multifasciata	5	1	20
Orchesella villosa	1	0	—
Hypogastrura viatica	0	1	—

Discussion

This paper reports the first field results obtained by using PCR primers to amplify the DNA of any prey species extracted from the guts of any arthropod predator, where both predators and prey were natural, unmanipulated field populations. The only comparable study is that by Hoogendoorn & Heimpel (in press), who introduced European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), egg masses pinned on cards to corn plants. Predators, coccinellid beetles, were later collected and six out of 562 were found to test positive using primers published in Hoogendoorn & Heimpel (2001). Our study of predation on Collembola is also the first study to develop primers for the detection of nonpest prey. Although several recent laboratory-based studies have shown that this PCR-based approach may be feasible, none has gone on to demonstrate that this is a practical means of recording predation simultaneously on a range of different prey in the field. Until now the primary technique for studying such predation relied upon the development of prey-specific monoclonal antibodies (reviewed in Symondson 2002).

Three species-specific markers were successfully developed for the detection of predation on three common species of Collembola. None cross-reacted with anything else. Primers to parts of the COI mitochondrial gene again proved to be useful for obtaining species-level discrimination (Agustí *et al*. 2003). Much is known about this gene, which is widely used in phylogenetic studies. Different parts of the gene evolve at different rates and conserved primers that amplify the whole, or appropriate regions, of the gene are available (Folmar *et al*. 1994; Simon *et al*. 1994; Lunt *et al*. 1996). Targeting mtDNA ensured that there were multiple copies of the gene per insect cell, enhancing detectability. The feeding trial demonstrated that a single collembolan could be detected within a spider for at least 24 h in 100% of the predators tested. This compares well with previous studies where similar periods of detection were obtained (Zaidi *et al*. 1999; Agustí *et al*. 2003). Long detection periods have been demonstrated for prey proteins in spiders using antibodies (Harwood *et al*. 2001c). One advantage of primers to detect DNA is that the rates at which the target sequences break down during digestion are likely to be far less variable than is the case for different protein epitopes. The latter may be either labile or refractory

to digestion, resulting in detection periods varying from almost instantaneous denaturing to survival for many days (Symondson 2002). The DNA fragments we amplified varied from 211 to 276 bp in length and it has been shown that sequence length can affect detection times (as discussed earlier). Interestingly, the *Isotoma anglicana* fragment was the longest (276 bp), and hence should have broken down the most rapidly, suggesting that predation may have been relatively more important on this species than our data suggest. Nobody has yet studied whether, for example, AT-rich DNA breaks down during digestion more rapidly than CG-rich DNA; it is possible that the extra hydrogen bonds could make the latter more resistant. Similarly the digestion of cell walls and the release of DNA could vary between prey species. Neither of these scenarios are likely where the prey are closely related (as here) and the primers are targeting the same region of the same gene.

Apart from a laboratory study by Greenstone & Shufran (2003), which demonstrated that DNA from a single aphid could be detected in the guts of spiderlings for up to 12 h using primers described in Chen *et al*. (2000), the work we report here is the first to use DNA-based techniques to study predation by spiders. The field results demonstrated high levels of predation on Collembola. Linyphiid spiders have been shown to locate their webs in areas of high Collembola density in the field and these invertebrates represent a potentially major food resource (Harwood *et al*. 2001a, 2003). Increased density of Collembola, in response to the addition of detritus, can lead to increased numbers of spiders and other predators (Halaj & Wise 2002), while spider removal can cause Collembola numbers to increase (Lawrence & Wise 2000). However, it has been difficult until now to determine whether a particular Collembola species was being exploited to a greater degree than others in the field. The factors that contribute to prey choice are often complex (Symondson *et al*. 2002). Our results clearly showed preference for *I. anglicana*; few of these Collembola were found in the mesocosms and yet a high percentage of the spiders had consumed this species (Table 3). It is possible that such preference was, at least in part, responsible for the low density of this species of Collembola in the plot. *Isotoma anglicana* have been shown in laboratory feeding trials to be a high-quality food for spiders (Marcussen *et al*. 1999). Conversely, the most common species of Collembola, *Lepidocyrtus cyaneus*, was eaten to a significantly lesser extent, much less than might have been expected from the densities recorded in the mesocosms (Table 3). In previous work, Alderweireldt (1994) showed that even where *Lepidocyrtus* spp. were abundant in the field they were rarely eaten by linyphiid spiders, and this was confirmed in the laboratory where *Isotomurus pallustris* (Müller) were consumed in clear preference to *Lepidocyrtus* spp. There appear to be no suggestions in the literature that *L. cyaneus* contains toxins or antifeedants, unlike, for example, the highly toxic *Folsomia candida* Willem (Toft & Wise 1999; Oelbermann & Scheu 2002). However, many species of Collembola, including *L. cyaneus* and *Entomobrya multifasciata* but not *I. anglicana*, possess scales. Such scales have been shown to help Collembola escape from predatory beetles (Bauer & Pfeiffer 1991) while scales help many insects escape from spiders' webs (Nentwig 1982). It is possible that these scales are more of a hindrance to predation by male spiders than by the larger females (Table 2), although feeding by mature males of several species of linyphiid can in any case be minimal (Alderweireldt 1994). Another factor may have been prey size. Adult *I. anglicana* (4 mm) are more than three times the size of *E. multifasciata* (1.5 mm) and *L. cyaneus* (1.2 mm) and may have been more apparent to the predators. Alderweireldt (1994) found a preference for larger Collembola species by linyphiid spiders in laboratory studies. Other factors that might affect prey choice include vertical stratification of different Collembola species within the soil (Faber & Joosse 1993) affecting encounter rates with spiders (although the Collembola targeted in our study are all considered to be primarily surface-active species). Similarly, different Collembola species have different diel activity patterns and responses to temperature (Frampton *et al*. 2001), which will interact in complex ways with the hunting strategies and activity patterns of different species of spider, again affecting encounter rates. For whatever reason, the spiders showed strong preference for *I. anglicana*, and cultural techniques that might enhance densities of this species could help to increase spider numbers and hence control of pests such as aphids.

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This study was primarily a collaboration between postdoctoral fellow Nuria Agustí and Bill Symondson, conducted while the former was working with Bill Symondson's group at Cardiff University. Both Nuria Agustí and Bill Symondson have worked for many years on the development of novel methods of detecting and quantifying prey remains in predators. The field study was part of a long-term collaboration between Bill Symondson and senior entomologist Keith Sunderland at HRI-Wellesbourne, studying spider ecology in agroecosystems. The Collembola were identified by postdoc James Harwood, while the spiders were collected and identified by PhD student Simon Shayler. The field data were analysed by PhD student Ian Vaughan.
