Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field

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Abstract

A molecular approach, using aphid-specific monoclonal antibodies, was used to test the hypothesis that alternative prey can affect predation on aphids by linyphiid spiders. These spiders locate their webs in cereal crops within microsites where prey density is high. Previous work demonstrated that of two subfamilies of Linyphiidae, one, the Linyphiinae, is web-dependent and makes its webs at sites where they were more likely to intercept flying insects plus those (principally aphids) falling from the crop above. The other, the Erigoninae, is less web-dependent, making its webs at ground level at sites with higher densities of ground-living detritivores, especially Collembola. The guts of the spiders were analysed to detect aphid proteins using enzyme-linked immunosorbent assay (ELISA). Female spiders were consuming more aphid than males of both subfamilies and female Linyphiinae were, as predicted, eating more aphid than female Erigoninae. Rates of predation on aphids by Linyphiinae were related to aphid density and were not affected by the availability of alternative prey. However, predation by the Erigoninae on aphids was significantly affected by Collembola density. Itinerant Linyphiinae, caught away from their webs, contained the same concentration of aphid in their guts as web-owners. However, nonweb-owning Erigoninae, living away from Collembola aggregations at web-sites, contained significantly higher concentrations of aphid. For both subfamilies there was evidence of a disproportionate increase in predation on aphids once Collembola populations had declined. It was concluded that nonaphid prey, by helping to maintain spiders in the field, can significantly affect predation on aphids.

Keywords: ELISA, gut content analysis, monoclonal antibodies, niche axes, prey choice, web-location

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Introduction

Harwood *et al.* (2001a, 2003) demonstrated that spiders in winter wheat fields locate their webs nonrandomly in relation to prey availability. They showed that the spiders were not simply responding to the same microhabitat cues as the prey, but that each subfamily of spider was aggregating to microsites where they were most likely to find prey that were vulnerable to their different hunting strategies (Harwood *et al.* 2003). Thus, more ground-living prey,

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such as Collembola, were found at the web-sites of the Erigoninae, which make their webs on or close to the ground and are less web-dependent, frequently hunting away from their webs (Sunderland *et al.* 1986; Alderweireldt 1994). Conversely, more aerial prey, such as aphids and Thysanoptera, were found at the web-sites of the Linyphiinae, which make their webs a few centimetres higher up the wheat stems and are more web-dependent, rarely moving away from their webs (Sunderland *et al.* 1986; Alderweireldt 1994). The data supported the hypothesis that web-sites of different coexisting species of spider were analogous to diversity within plant communities. Community diversity may be maintained primarily by an equilibrium model in which each spider constructs its webs within different microsites, constrained by relatively narrow niche axes

(Harwood *et al.* 2003). Despite being generalists, the spiders were not aggregating to the same resources.

In these earlier studies mini-sticky traps and mini-quadrats were deployed at spider web-sites to measure prey availability. However, the presence of prey at web-sites does not necessarily mean that it was exploited by the predators. In order to confirm our hypotheses and prove that the different spider subfamilies were not simply aggregating to microsites where different prey were to be found, but were consuming prey in the proportions predicted from their hunting strategies, we needed to use a molecular approach.

In another recent study we used polymerase chain reaction (PCR) primers, designed to amplify DNA from three species of Collembola, to compare the availability of the different Collembola species at the field sites where the spiders were captured, with the frequency with which we could detect each species in the guts of the spiders (Agustí *et al.* 2003). We demonstrated clearly that the spiders (Linyphiidae) were exercising strong prey choice. The species of Collembola that was least numerous in the field, *Isotoma anglicana* (Lubbock) (Collembola: Isotomidae), was eaten more frequently than two more numerous Collembola species. *I. anglicana* has been shown in laboratory studies to be capable of promoting spider fitness (Marcussen *et al.* 1999). Prey choice is a combined measure of many other factors, including encounter rates between predator and prey and the ability of the prey to resist attack, but in this instance a highly nutritious prey was selected, suggesting an optimal foraging paradigm.

In the experiments reported here the aim was to compare aphid availability at the web-sites of Linyphiinae and Erigoninae, with aphid remains in the guts of the spiders removed from the web-sites monitored by Harwood *et al.* (2003). We did this by measuring aphid ingestion, using an aphid-specific monoclonal antibody (Symondson *et al.* 1999) to analyse the guts of the spiders, allowing us to compare aphid consumption with both aphid availability at web-sites and the availability of alternative prey. Although the initial costs of producing a new monoclonal line are high (Chen *et al.* 2000), once created these antibodies can be replicated in limitless quantities and can be used to screen field samples more rapidly and inexpensively than using DNA-based approaches (Symondson 2002). This particular antibody has been shown to be capable of detecting aphid remains in carabid beetles and linyphiid spiders for more than a week (Harwood *et al.* 2001b), far longer than has been achieved so far for the detection of prey DNA (Zaidi *et al.* 1999; Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Agustí *et al.* 2003; Cuthbertson *et al.* 2003; Greenstone & Shufran 2003; Sheppard *et al.* 2004).

A number of hypotheses were tested, relating to prey choice in the field, that could only be addressed using a molecular approach. The first hypothesis was that aphids would be consumed in the proportions found at web-sites in the field. If this proved to be correct then it would suggest that aphids are a major component of the diet of the spiders and that aphid consumption rates were relatively insensitive to changes in alternative prey densities. Nutrition (which includes avoidance of excessive prey toxins) may play a significant part in prey choice by spiders (Greenstone 1979; Toft 1997; Toft & Wise 1999). Many aphids are known to contain toxins, and predators fed on an exclusive diet of aphids soon die and produce few if any offspring (Sunderland *et al.* 1996; Toft 1997; Bilde & Toft 2001). As these spiders are generalist predators we further hypothesized that alternative prey abundance and biomass should also affect aphid consumption. However, it was demonstrated in Harwood *et al.* (2003) that, of the two spider subfamilies, the Linyphiinae were more web-dependent, locating their webs where more aphids were present, whereas the Erigoninae were less web-dependent, locating their webs where more ground-living prey were available, especially Collembola. We therefore hypothesized that a higher proportion of the Linyphiinae should contain the remains of aphids in their guts than the Erigoninae, and further that the presence of Collembola might be expected to have a significant negative effect on aphid predation by the latter subfamily but not the former.

Harwood *et al.* (2003) also showed that spider weight in the field was a good surrogate measure of their state of nutrition. Spiders experience high levels of competition for web-sites (Samu *et al.* 1996; Heiling & Herberstein 1999; Riechert & Hall 2000) and readily abandon patches or webs if their current location is not perceived as providing sufficient numbers of prey (Riechert 1976; Janetos 1982; Olive 1982; Persons & Uetz 1996, 1997, 1998). Spiders at web-sites are therefore more likely to feed on an elevated abundance and diversity of prey (Harwood *et al.* 2001a, 2003) and this mixed diet would translate in generalist predators to improved survival, development and reproduction (Greenstone 1979; Toft & Wise 1999; Mayntz & Toft 2001). We would therefore expect nonweb-owning spiders (that are likely to have abandoned a poor quality web-site or been evicted from a good one) to have fed on fewer and/or nutritionally poorer quality prey. The results showed that among the web-dependent Linyphiinae, in particular *Tenuiphantes tenuis* (Blackwall) (Araneae: Linyphiidae), spiders caught away from their webs weighed less than those collected from web-sites. This did not apply to the less web-dependent Erigoninae. This difference between the subfamilies may have been a result of their hunting strategies in relation to aphid availability and was examined here by testing the hypothesis that aphids were an equally important part of the diets of web-owning spiders and those without webs.

It has been suggested that spiders and other predators may be most effective at limiting numbers of aphids in a crop early in the year, when aphid densities are still low

(Chiverton 1987; Landis & Van der Werf 1997). Predation theory has shown that generalist predators may be at least as effective as specialist predators and parasitoids at controlling pest species specifically because they (unlike the specialists) can exist on nonpest prey and are thus already in the field, ready to attack the pests early in the season (Chang & Kareiva 1999; Symondson *et al.* 2002). Such 'lying-in-wait' strategies can even, in theory, drive a prey species to extinction without adversely affecting the predators (Murdoch *et al.* 1985). Harwood *et al.* (2003) demonstrated that early in the season Collembola densities were high, providing an ideal alternative prey to spiders which are known to feed on Collembola (Sunderland *et al.* 1996; Marcussen *et al.* 1999; Agustí *et al.* 2003). Settle *et al.* (1996) demonstrated in a rice ecosystem that predators can build up their numbers early in the season by feeding on detritivores. When the latter declined as the crop matured, the predators were present in high numbers and therefore were able to exert enhanced control of invading pests. We tested the hypothesis that, as Collembola numbers declined, predation on aphids would increase at a rate that was greater than expected from the rate of aphid population growth. Any such change would confirm that Collembola densities were affecting temporal change in predation by spiders on aphids.

Aphids cause extensive damage to wheat crops throughout the world by direct feeding (Fletcher & Bardner 1969; Vickerman & Wratten 1979; Oakley *et al.* 1993) and the transmission of barley yellow dwarf virus (Oswald & Houston 1951, 1953), the most important virus affecting cereals (Araya *et al.* 1996). The long-term aim of this study was therefore to examine the importance of linyphiid spiders as natural enemies of aphids and, more specifically here, the effect of alternative prey on the dynamics of spider–aphid interactions.

Methods

An aphid-specific monoclonal antibody, MdW-7(1)G1 (Symondson *et al.* 1999), was used to test field-collected spiders for the presence of aphid proteins. Laboratory feeding trials at 16 °C indicated this antibody has a detection time for the aphid *Sitobion avenae* (F.) (Homoptera: Aphididae) of up to 193 h in female and 169 h in male *T. tenuis* (Harwood *et al.* 2001b).

Antibody characterization

Symondson *et al.* (1999) tested this antibody against 33 species of invertebrates including arthropods, annelids and molluscs. Here a further 38 arthropods were assayed, representing spider predators and their prey from the field site at Horticulture Research International, Wellesbourne, UK. Such site-specific screening is essential whether molecular assays are based upon antibodies or PCR.

All invertebrates were screened by indirect enzyme-linked immunosorbent assay (ELISA) using 96-well micro-titration plates (Falcon Pro-Bind Assay Plates, Becton Dickinson Labware, Oxford, UK). The indirect ELISA followed a standardized protocol (e.g. Symondson & Liddell 1996). All invertebrates were homogenized 1:20 (w/v) separately in phosphate buffered saline (PBS), pH 7.4. The homogenate was dispersed for 1 min on a vortex mixer and centrifuged at 8000 *g* for 15 min at room temperature. The particulate matter was discarded and the supernatant transferred to clean 0.5 mL Eppendorf tubes and stored at −20 °C.

Each ELISA plate was coated with a $1.5 \times$ dilution series of aphid (*S. avenae*) standards that provided absorbance readings for aphid protein concentrations from 265.5 to 4.6 ng 200 μL^{-1}, as well as two negative controls. Each aphid standard was added to two ELISA plate wells and screened by indirect ELISA (described below). Protein concentrations were calculated using the Bio-Rad Protein Assay System (Bio-Rad Laboratories Inc., Hemel Hempstead, UK). In order to keep protein concentrations constant throughout the dilution series, the aphid standards were diluted with heterologous protein (Symondson & Liddell 1995) such that each sample contained the same total concentration of protein. The heterologous proteins were a homogenized mixture of starved linyphiid spiders.

Non-target prey and starved predators (10 replicates of each species) were screened against this antibody to ensure specificity. The supernatants were diluted to a final concentration of 1:20 000 (w/v) in PBS. Each sample was added to two ELISA plate wells, at 200 μL per well, and left to incubate overnight at room temperature. All wells of the ELISA plate were washed three times with PBS-Tween (0.05% Tween 20) (Sigma-Aldrich, Poole, UK). The anti-aphid monoclonal antibody, MdW-7(1)G1, was diluted 1:4000 in PBS-Tween and 200 μL added to one ELISA plate well for each invertebrate sample. In parallel, 200 μL of PBS-Tween was added to the duplicated invertebrate sample on the ELISA plate. The samples were allowed to incubate for 2 h at room temperature and plates were then washed three further times with PBS-Tween. All wells of the ELISA plates were coated and incubated for 1 h with Immuno-Pure® goat anti-mouse IgG horseradish peroxidase enzyme conjugate (Pierce, Rockford, IL, USA) diluted 1:4000 in PBS-Tween. Plates were washed a further three times with PBS-Tween. The enzyme substrate, *o*-phenylenediamine in a citrate-phosphate buffer, was added at 200 μL per well to all wells and placed in the dark for 30 min to allow colour development. The reaction was stopped by adding 50 μL of 2.5 M sulphuric acid. The absorbance was recorded at 492 nm (OD 492) using an ELISA plate spectrophotometer (Thermomax Plate Reader, Molecular Devices, Sunnyvale, CA, USA). Absorbance readings for duplicated wells containing no antibodies were subtracted from wells

containing antibody to eliminate the effects of nonspecific binding (Symondson *et al.* 2000).

Detection period within Erigone atra *(Blackwall)*

In an earlier study (Harwood *et al.* 2001b) the decay rate of aphids during digestion in the guts of *T. tenuis* were measured using the same monoclonal antibody as that used in the current study. As decay rates can vary considerably between predator species (e.g. Symondson & Liddell 1993a), and this has an effect on the proportion of predators that test positive in an ELISA, it was necessary to repeat these experiments for the other spider species found at our field site. The protocols employed were identical to those used in Harwood *et al.* (2001b).

E. atra (Araneae: Linyphiidae) were collected from fields of winter wheat and maintained at 16 °C on a 16:8 light:dark cycle. Spiders were housed separately in 50 × 15 mm triple-vented Petri dishes containing a damp plaster of Paris and charcoal base to ensure high humidity. *E. atra* were fed for approximately 1 month on a diet of live *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) followed by a 2-week starvation period. Sixteen starved spiders (eight female and eight male) were frozen as starved controls. The remaining spiders were allowed to feed *ad libitum* on live *S. avenae* for 2 h and any nonfeeding individuals excluded. Sixteen spiders (eight female and eight male) were killed by freezing immediately after the feeding period. Further spiders were frozen at 2, 4, 8, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h after feeding, 16 individuals (eight female and eight male) at each time period. Frozen spiders were transferred to clean 1.5 mL Eppendorf tubes and stored at −20 °C. All samples were prepared as described above and screened by indirect ELISA.

Less abundant species of linyphiid spider, *E. dentipalpis* (Wider), *Bathyphantes gracilis* (Blackwall), *Oedothorax* spp. and *Meioneta rurestris* (C.L. Koch) (Harwood *et al.* 2001a, 2003) were collected, stored and fed under identical conditions to those described for *E. atra*. Eight female spiders of each species were frozen 0 h, 24 h and 48 h after feeding on live *S. avenae*.

Field experiment

Spiders were collected by pooter from webs and on the ground within four winter wheat fields at HRI Welles-bourne over the summer. In parallel with the collection of spiders, mini-sticky traps and mini-quadrats (see below) were used to monitor the availability of potential prey to linyphiids (Harwood *et al.* 2001a, 2003).

Spiders were located at random from within webs ('web-owners') or on the ground away from the web ('nonweb-owners') by collecting the first spider observed at an undisturbed location within the crop. The sampling methods, mini-sticky traps and mini-quadrats, were designed to monitor the availability of prey to linyphiid spiders and were undertaken at the time when the spiders were collected from the field. The two sampling techniques were designed to monitor linyphiid prey availability at two different microhabitat scales. Mini-sticky traps (area ~ 7.5 cm²) represented a cumulative record of prey entering the web-site of spiders over time (traps were left *in situ* for 24 h) while larger mini-quadrats (area ~78.5 cm²) provided an instantaneous measure of arthropod abundance. The mini-quadrats enabled less active species, including those hiding under stones and vegetation, to be collected. Sampling was carried out between late April and harvest in late July (60 sticky trap and 60 quadrat samples were taken on each sampling occasion). Following the collection of each spider, all individuals were placed in separate Eppendorf tubes on ice and transferred to a −20 °C freezer within 1 h of collection. Each spider was subsequently identified, weighed to the nearest 0.01 mg, diluted 40 × (w/v) in PBS and homogenized separately. Supernatants were stored at −20 °C and screened by indirect ELISA. It has been suggested that food-chain errors can arise during post-mortem gut-content analyses due to secondary predation and scavenging (Sunderland 1988, 1996; Symondson 2002). Despite these potential sources of error, within the current spider–aphid system such errors are unlikely, given the insignificant detection of aphid proteins following secondary predation (Harwood *et al.* 2001b; using the same monoclonal antibody as here) and the low numbers of dead aphids falling from crops (Winder *et al.* 1994).

The concentration of antibody-recognizable aphid protein within spiders was calculated from the protein standards on each plate using regression analysis. The quantity of aphid protein (Q) present within the spider was calculated using the following formulae (after Symondson *et al.* 2000):

$$Q = \frac{\{([B \times 20\,000]/200) \times S\}}{1000}\mu g$$

where B is the biomass of spider (in mg) and S is the concentration of antibody-recognizable aphid protein per 200 μL (i.e. the quantity of aphid protein within the ELISA plate well). In this equation, the spider biomass (B) was multiplied by the ELISA dilution rate (20 000) and then divided by the volume of diluted sample added to each ELISA plate well (200). Thus, the equation simplifies to:

$$Q = (B \times S)/10\ \mu g.$$

Statistical analysis

Prior to analyses spiders were separated on the basis of subfamily (Erigoninae or Linyphiinae) and gender. In order to stabilize variances, data were log-transformed when necessary and the proportions of spiders testing positive

(i.e. those assayed by ELISA and found to contain a concentration of antibody-recognizable aphid protein above the significance level) were arcsine-transformed prior to analysis. Regression analyses were performed using means (aphid concentrations and quantities, invertebrate numbers and biomass) for each field and date. This allowed us to use data for spiders at web-sites where there were no sticky trap data (in Harwood *et al.* 2003 half the spider web-sites were monitored using sticky traps and half using mini-quadrats).

Results

Antibody characterization

Cross-reactivity on the 38 invertebrates (Table 1) showed that the highest level of cross-reactivity was with field-collected Delphacidae, although the aphid protein concentration equivalent was below that for *Trialeurodes vaporariorum* (Westwood) (Symondson *et al.* 1999). Given that *T. vaporariorum* was not present at our field site (Harwood *et al.* 2001a, 2003), the cross-reaction significance level, above which a sample is deemed to contain aphid proteins [defined as the mean + 2.5 standard deviation (SD) aphid protein concentration equivalent for the non-aphid invertebrate giving the strongest reaction in an ELISA: Symondson & Liddell 1993a], was 11.2 ng 200 μL^{-1} using the mean aphid protein equivalent for field-collected delphacids.

Decay of aphid remains within the guts of spiders

Given that the consumption of aphids by spiders could have occurred at any time during the 2 h feeding period, consumption was assumed to have taken place at the midpoint (after 1 h) and thus 1 h has been added to each time interval (Symondson & Liddell 1995). The absorbance readings for detection of the epitope to which this particular antibody binds first increased with time and then declined (Symondson *et al.* 1999; Harwood *et al.* 2001b) (Fig. 1). This is thought to be caused by exposure of additional internal antibody-binding sites within the target protein molecules during digestion. The rate at which aphid protein decayed within the guts of these spiders showed a linear relationship between 5 h and 169 h after feeding, for both female and male spiders (Fig. 1). Using the regression equations, the antigenic half-life (defined as the time taken for antigen concentration to decline to half its original level) was 99.4 h in females and 98.6 h in males, comparable to the 101.1 h in female and 105.4 h in male *T. tenuis* (Harwood *et al.* 2001b). Analysis of covariance indicated that there was no significant difference between the rate at which the slopes for the two sexes decline ($F_{1,14} = 0.15$, $P > 0.05$) or the *y*-axis intercepts ($F_{1,14} = 0.31$, $P > 0.05$).

The aphid protein equivalents for six species of spider measured at three postfeeding time intervals are presented

Table 1 Level of cross-reactions between the MdW-7(1)G1 monoclonal antibody and a range of invertebrate predators and prey. Values are expressed as *Sitobion avenae* protein equivalents ± SE (*n* = 10). Readings in subsequent assays of field-caught spiders that were > 11.2 ng *S. avenae* protein equivalent were considered positive for aphid consumption; see text for calculations

| Invertebrates	*S. avenae* protein equivalent (ng 200 μL^{-1})
Collembola	
Isotoma anglicana (Lubbock)	3.23 ± 0.41
Isotomurus palustris (Müller)	3.64 ± 0.33
Lepidocyrtus cyaneus Tullberg	2.89 ± 0.21
Entomobrya multifasciata (Tullberg)	2.63 ± 0.26
Folsomia candida Willem	2.05 ± 0.18
Hymenoptera	
Aphidius rhopalosiphi De Stephani-Perez	2.01 ± 0.17
Lasius niger L.	4.02 ± 0.39
Coleoptera	
Aleocharinae spp.	0.47 ± 0.03
Tachyporus hypnorum F.	0.82 ± 0.06
Tachyporus chrysomelinus L.	0.73 ± 0.04
Staphylinus olens Müller	0.63 ± 0.05
Tribolium castaneum Herbst	0.31 ± 0.04
Coccinella septempunctata L.	0.71 ± 0.08
Notiophilus spp.	0.42 ± 0.04
Atomaria spp.	0.24 ± 0.02
Enicmus spp.	0.21 ± 0.03
Thysanoptera	
Frankliniella occidentalis (Pergande)	4.51 ± 0.67
Hemiptera	
Delphacidae spp.	9.03 ± 0.42
Nilaparvata lugens Stal	8.94 ± 0.54
Philaenus spumarius (L.)	4.37 ± 0.44
Berytinus minor Herrich-Schaeffer	6.69 ± 0.48
Rhodnius prolixus Stal	7.02 ± 0.54
Lepidoptera	
Plodia interpunctella (Hubner)	1.03 ± 0.23
Neuroptera	
Chrysoperla carnea Stephens	0.67 ± 0.08
Isopoda	
Oniscus asellus L.	0.89 ± 0.08
Dictyoptera	
Blatella germanica (L.)	2.30 ± 0.21
Acari	
Acari spp.	0.54 ± 0.03
Araneae	
Erigone atra (Blackwall)	1.23 ± 0.09
Erigone dentipalpis (Wider)	1.56 ± 0.19
Tenuiphantes tenuis (Blackwall)	2.16 ± 0.12
Bathyphantes gracilis (Blackwall)	1.97 ± 0.14
Meioneta rurestris (C. L. Koch)	1.59 ± 0.10
Oedothorax apicatus (Blackwall)	2.03 ± 0.23
Oedothorax retusus (Westring)	2.19 ± 0.19
Oedothorax fuscus (Blackwall)	2.00 ± 0.17
Pachygnatha degeeri Sundevall	1.49 ± 0.16
Pardosa agrestis (Westring)	2.68 ± 0.28
Pardosa hortensis Thorell	2.76 ± 0.31
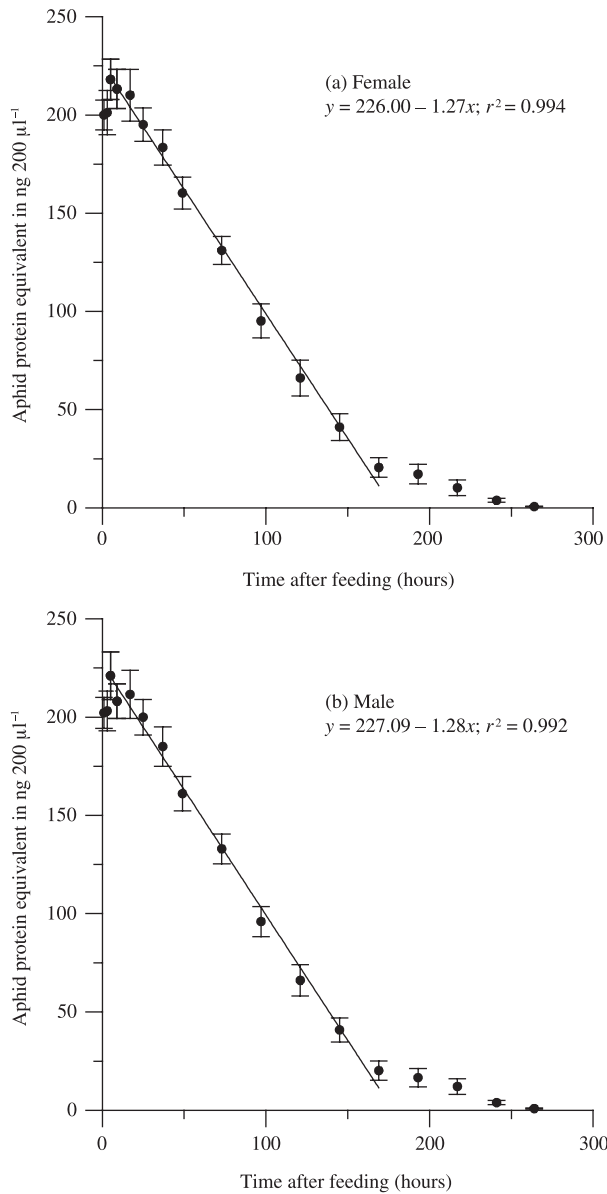
Fig. 1 Relationship between *Sitobion avenae* protein concentration equivalents in the guts of (a) female and (b) male *Erigone atra* and time. Regression line shows linear relationship between 5 and 169 h after feeding. Bars are ± SE.

in Table 2. Analysis of variance (ANOVA) indicated no significant difference between the amounts of detectable aphid antigen in any of the species tested ($F_{5,126} = 0.24$, $P > 0.05$) at any time period but detectable antigen declined with time ($F_{2,126} = 61.04$, $P < 0.001$).

Aphid consumption in the field

Gut-content analysis showed that many spiders contained a significant concentration of antibody-recognizable aphid protein within their guts (Linyphiinae females 28.6%, males 18.4%; Erigoninae females 25.4%, males 17.6%). These
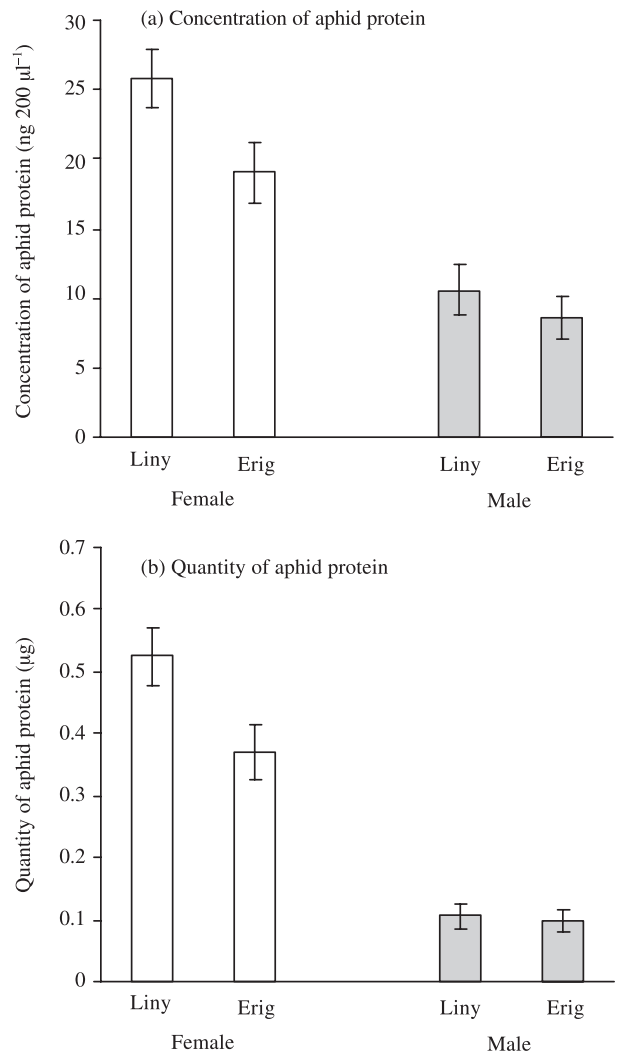

Fig. 2 Differences between (a) concentration and (b) quantity of antibody-recognizable aphid protein (at ELISA dilutions) in female and male Linyphiinae vs. Erigoninae. Bars are ± SE.

differences between subfamilies in the proportions that tested positive were not significant, either for males ($F_{1,54} = 0.84$, $P = 0.364$) or females ($F_{1,54} = 0.50$, $P = 0.481$). Significantly more female spiders contained aphid protein concentrations above the cross-reaction significance threshold than males (Linyphiinae: $F_{1,54} = 5.67$, $P = 0.021$; Erigoninae: $F_{1,54} = 5.04$, $P = 0.029$).

However, we had shown previously that greater numbers of aphids were captured on sticky traps at the web-sites of the Linyphiinae compared with the Erigoninae, and interpreted this as a consequence of their different web-locations and foraging strategies (Harwood *et al.* 2003). The ELISA results showed that female Linyphiinae were also consuming more aphids, and contained a significantly greater concentration ($F_{1,1019} = 4.52$, $P = 0.034$) and quantity ($F_{1,1019} = 4.85$, $P = 0.028$) of aphid protein within their guts compared to female Erigoninae (Fig. 2). However, no

	Aphid protein equivalent in ng 200 μL⁻¹		
Species	After 0 h	After 24 h	After 48 h
Erigone atra	200.05 ± 8.14	195.11 ± 8.30	160.33 ± 8.14
Erigone dentipalpis	201.11 ± 8.52	196.08 ± 4.65	165.90 ± 6.08
Tenuiphantes tenuis	196.12 ± 8.71	194.86 ± 5.87	162.39 ± 9.37
Bathyphantes gracilis	200.02 ± 8.50	195.36 ± 6.50	159.43 ± 8.68
Meioneta rurestris	201.74 ± 5.55	199.62 ± 6.49	159.43 ± 8.68
Oedothorax spp.	202.02 ± 5.23	200.49 ± 8.79	158.23 ± 4.78

Table 2 Mean (± SE) aphid protein equivalents for six species of linyphiid spider fed *ad libitum* on *Sitobion avenae* for 2 h. Spiders were killed by freezing 0 h, 24 h and 48 h after feeding. Only female spiders were included in the analysis and *n* = 8 for each time period for each species
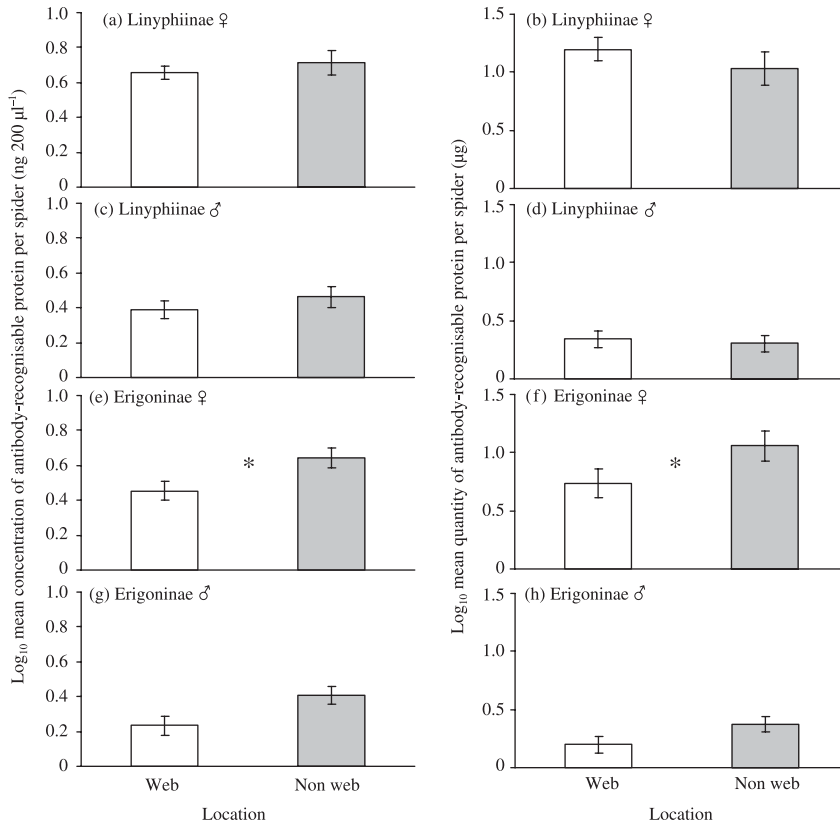

Fig. 3 Antibody-recognizable aphid proteins (at ELISA dilutions) found within the guts of Linyphiinae and Erigoninae captured at web-sites or away from the web. Results are presented separately for the concentration and quantity of aphid protein within the different subfamilies and gender of spider. Bars are ± SE. Level of significance between bars indicated on figure: *$P < 0.05$.

such differences were found for males (concentration: $F_{1,520} = 0.65$, $P = 0.422$; quantity: $F_{1,520} = 0.05$, $P = 0.831$).

Spiders were collected from webs and areas away from the web. Linyphiinae at the two locations did not differ in the concentration (female: $F_{1,645} = 1.80$, $P = 0.180$; male: $F_{1,265} = 0.96$, $P = 0.328$) or quantity (female: $F_{1,645} = 0.73$, $P = 0.394$; male: $F_{1,265} = 0.13$, $P = 0.721$) of aphid proteins in their guts (Fig. 3). Interestingly, however, among the less web-dependent Erigoninae, the concentration of aphid protein was greater in spiders captured away from webs (female: $F_{1,372} = 5.93$, $P = 0.015$; male: $F_{1,253} = 4.42$, $P = 0.037$), indicating a relatively higher utilization of aphids by nonweb-owners. However, the calculated quantity of aphid material within the guts of nonweb-owners was not significantly greater than those individuals captured at web-sites

(female: $F_{1,372} = 3.19$, $P = 0.075$; male: $F_{1,253} = 2.79$, $P = 0.096$) (Fig. 3).

As expected, female spiders contained a greater quantity of aphid protein within their guts than males due to their larger size (Linyphiinae: $F_{1,910} = 33.02$, $P < 0.001$; Erigoninae: $F_{1,625} = 25.28$, $P < 0.001$) (Fig. 3). However, the concentration of aphid protein in females was also higher, indicating that aphids constituted a more significant component of the diet of female linyphiid spiders (Linyphiinae: $F_{1,910} = 16.51$, $P < 0.001$; Erigoninae: $F_{1,625} = 16.11$, $P < 0.001$) (Fig. 3).

The concentration of aphid protein present within spiders changed significantly between weeks for both Erigoninae (female, $F_{6,367} = 26.18$, $P < 0.001$; male, $F_{6,248} = 9.75$, $P < 0.001$) and Linyphiinae (female, $F_{6,640} = 36.22$, $P < 0.001$;
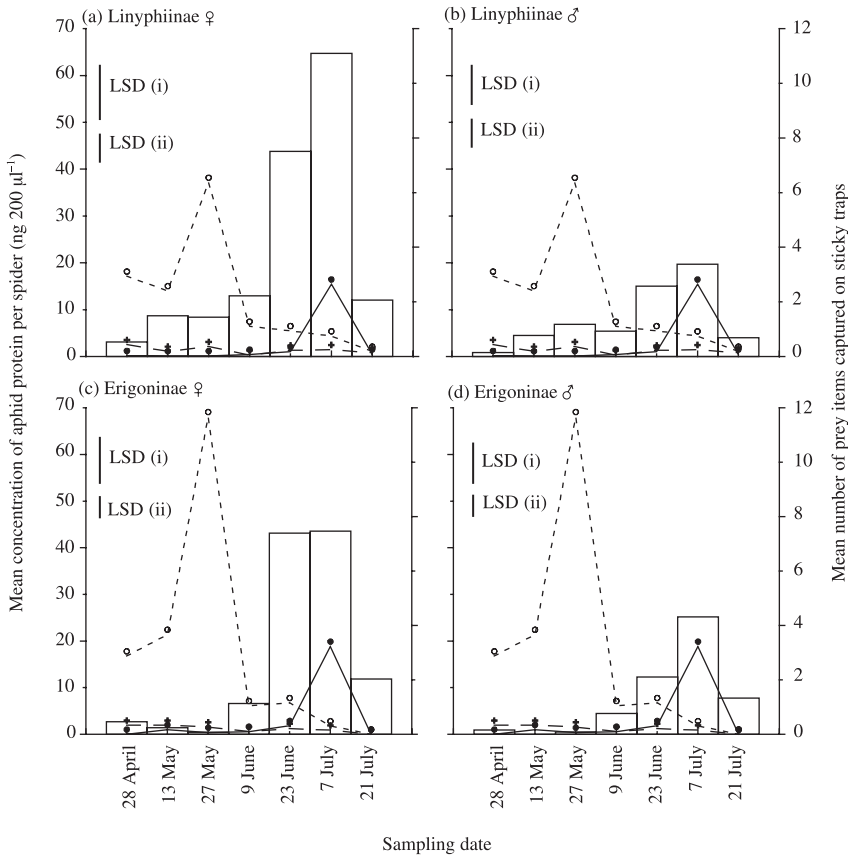
Fig. 4 Temporal changes in concentration of antibody-recognizable aphid protein within spiders (bars), number of Collembola (○), number of aphids (●) and number of Diptera (+) within four fields of winter wheat. Solid bars represent (i) LSD for comparison of the weekly variation in concentration of aphid protein within spider guts and (ii) LSD for comparison of the weekly variation in number of aphids captured on mini-sticky traps. Data are presented as means per sampling date, pooled for all fields.

male, $F_{6,260} = 3.56$, $P < 0.001$), with greatest consumption during late June and early July (Fig. 4). It is interesting to note that, for female spiders in both subfamilies, once the early-season high density of Collembola declined, aphid consumption increased rapidly, well before the considerable increase in aphid numbers recorded on 7 July. In addition, aphid consumption rose between 9 June and 23 June in the absence of any significant rise in aphid numbers (Fig. 4).

Aphid consumption by female spiders is clearly more significant than that by males (Fig. 4), and therefore regression analyses were performed on female data only. Analyses examined separately the relationship between aphid consumption and both aphid numbers and aphid biomass at spider web-sites. As r^2 values and significance levels were almost identical only the relationship with aphid numbers will be reported. There was a strong and positive relationship between aphid numbers at web-sites and the concentration (Erigoninae r^2 0.42, concentration = 34.1 + 8.74 $\log_e$ aphid number, $P < 0.001$; Linyphiinae r^2 0.45, concentration = 46.6 + 12.2 $\log_e$ aphid number, $P < 0.001$) and quantity (Erigoninae r^2 0.47, quantity = 0.669 + 0.179 $\log_e$ aphid number, $P < 0.001$; Linyphiinae r^2 0.39, quantity = 0.932 + 0.237 $\log_e$ aphid number, $P < 0.001$) of antibody-recognizable aphid remains in the spiders. Multiple regres-

sion models were then used to examine the effects of alternative prey on the spider–aphid interactions. The best-fitting models were with aphid concentrations in the guts of the spiders, which will be reported here, but, as in the regressions above, very similar results were obtained for aphid quantities. Concentrations of aphid in spiders were initially regressed against $\log_e$ aphid, $\log_e$ Collembola and $\log_e$ Diptera numbers. Diptera were in all cases nonsignificant and the regressions were repeated in the usual way without this factor. Collembola had a highly significant negative effect on the concentration of aphid in Erigoninae spiders (Table 3a). However, a similar analysis showed no effect of Collembola on the concentration of aphid in the guts of Linyphiinae (Table 3b); there was no evidence that alternative prey were affecting consumption of aphids which was strongly linked to aphid numbers at web-sites.

Discussion

These experiments represent the first use of a monoclonal antibody to study predation by spiders on aphids in the field and appear to be only the third use of monoclonal antibodies to study predation by spiders on anything outside the laboratory. An earlier study by Sigsgaard (1996) used a monoclonal antibody produced by Greenstone

Predictor	Coef.	SE coef.	T	P
(a) Erigoninae				
Regression equation: $\log_e$ concentration = 2.61 + 0.462 $\log_e$ aphids − 0.741 $\log_e$ Collembola				
Constant	2.6088	0.4333	6.02	0.000
$\log_e$ aphids	0.4624	0.1833	2.52	0.019
$\log_e$ Collembola	−0.7415	0.1856	−4.00	0.001
$S = 1.356$	$r^2 = 0.56$			
(b) Linyphiinae				
Regression equation: concentration = 46.8 + 12.4 $\log_e$ aphids + 1.00 $\log_e$ Collembola				
Constant	46.761	6.284	7.44	0.000
$\log_e$ aphids	12.420	2.840	4.37	0.000
$\log_e$ Collembola	1.001	3.450	0.29	0.774
$S = 19.26$	$r^2 = 0.45$			

Table 3 Multiple regression models of the effects of aphids and alternative prey on the concentration of aphid protein within (a) Erigoninae and (b) Linyphiinae spiders in winter wheat

& Trowell (1994) against *Helicoverpa* and *Heliothis* moths (Lepidoptera: Noctuidae) to study predation by spiders and other predators on *H. armigera* Hübner eggs in a crop of pigeonpeas in India. A second study by Ruberson & Greenstone (1998) found 25% of the spiders *Chiracanthium inclusum* Hentz (Araneae: Clubionidae) in a crop of cotton to be positive for heliothine egg proteins in their guts using the same antibody. Monoclonal antibodies are considered to be more specific (Greenstone 1996; Symondson 2002) than the polyclonal antisera that were deployed in earlier work on spider–aphid interactions (Chiverton 1987; Sunderland *et al*. 1987; Winder *et al*. 1994).

The rates at which the aphid proteins decayed and became undetectable during digestion were found to be the same for all species of spider tested. This was important because it allowed us to make direct comparisons between species and subfamilies. Previous studies have shown that different predators can have very different antigen digestion rates, even within the same family (Symondson & Liddell 1993b; Hagler & Naranjo 1997). Allowance must be made for any such difference when comparing numbers of field-collected predators testing positive. Quantities and concentrations of the aphid antigens detected were inevitably a combined measure of the amount eaten and the time since feeding. Models have been developed to translate such quantitative ELISA data into numbers of prey consumed (reviewed in Mills 1997), but even the most accurate of these, which incorporate a measure for antigen decay rate (Sopp *et al*. 1992), assume that prey are taken as discrete meals which are fully digested before another prey is consumed. The long detection periods found using this antibody and linyphiid spiders (Fig. 1) make such an assumption untenable, which is why we confine our analyses to detectable antigen concentration and biomass and make no attempt to assess the numbers of prey consumed.

Despite laboratory studies indicating that aphids are a poor quality nonpreferred prey item for generalist pred-

ators (Toft 1995; Bilde & Toft 2001) and that some spiders appear to develop aversions against these pests (Toft 1997), the field study indicated that aphids constituted a significant proportion of the diet of Linyphiidae. Using a sensitive and specific monoclonal antibody approximately 26% of female and 18% of male linyphiid spiders contained aphid protein. However, against expectation, there was no significant difference between the two subfamilies, in terms of the proportions of individuals that tested positive. Harwood *et al*. (2003) had found significantly more aphids at the web-sites of the Linyphiinae than the Erigoninae and thus the expectation was that more of the former would contain aphid remains. A later analysis showed that more Erigoninae captured away from web-sites tested positive than those that were web-owners. However, even when these itinerant spiders are excluded from the analysis the proportions of the two subfamilies testing positive were the same ($F_{1,54} = 0.49$, $P = 0.488$).

Overall, more female spiders contained aphid remains than males, which is significant from the point of view of potential effects on reproduction (see above). There were greater quantities of aphids in females (reflecting their greater size) but the fact that the concentrations of aphid in females were also greater suggests that they were choosing to incorporate a greater proportion of aphid in their diets than were the males. Female Linyphiinae contained higher concentrations and quantities of aphid than female Erigoninae, but there was no difference between the males. This suggests that the additional aphids at the web-sites of Linyphiinae were being exploited principally by the females.

Linyphiid spiders are not entirely confined to webs, even though some species favour a sit-and-wait hunting strategy. In fact, spiders readily abandon a patch or web if that microhabitat contains insufficient prey (Janetos 1982; Olive 1982; Persons & Uetz 1996, 1997, 1998) or if they are evicted from a good quality web-site through competition between individuals (Samu *et al*. 1996; Heiling & Herberstein 1999;

Riechert & Hall 2000), with the larger challenger almost always displacing the smaller occupant. Our hypothesis that aphid predation rates by web-owners and nonweb-owners would be the same was shown to be supported for the Linyphiinae but not the Erigoninae. Quantities and concentrations of aphid in male and female Linyphiinae were no lower for nonweb-owners. Linyphiinae are thought to be web-dependent (Alderweireldt 1994) and therefore the data suggest that either this is wrong, and they hunt actively for aphids on the ground when away from their webs, or (more likely) it is correct and the spiders are only away from their webs briefly, so that their gut contents reflect prey captured recently and consumed within recently vacated webs. By contrast, nonweb-owning Erigoninae contained significantly greater concentrations of aphid protein than individuals at web-sites (male and female). This may reflect the fact that Harwood *et al*. (2003) showed that Collembola densities at the web-sites of Erigoninae were high, certainly higher than at matched nonweb-sites. Agustí *et al*. (2003) demonstrated clearly that Collembola are an important prey for Erigoninae (more so than for Linyphiinae) and therefore web-owners could exploit this prey in preference to lower-quality aphids. Spiders without webs (through eviction or resource depletion at web-sites) may be exploiting more aphids through lack of choice. Although these nonweb-owners contained greater concentrations of aphid protein than web-owners, the calculated quantities were not (quite) significantly different.

Prey numbers trapped on sticky traps at web-sites varied considerably between weeks (Fig. 4). These data report activity–density, a combined measure that reflects more accurately availability of these prey to the spiders than would data on absolute densities. Aphids located higher up the plants would not be vulnerable to the species we studied, but the rates at which these aphids fall off the plants and onto sticky traps or webs will vary considerably and depend upon factors such as the wind, rain and activity of other predators such as ladybirds in the canopy (Sunderland *et al*. 1997; Losey & Denno 1999). In one study 35% of the tiller population of aphids fell from the crop canopy per day (Sunderland *et al*. 1986), while another showed that over 90% will return to the plant if not preyed upon (Sopp 1987). The prey must be active on the surface to be caught on sticky traps and hence in spiders' webs. There was a highly significant rise (15-fold increase at Linyphiinae web-sites and 10-fold increase at web-sites of Erigoninae) in aphid numbers on 7 July (Fig. 4). However, between late May and early July aphid numbers may be following an exponential rise that is typical of European aphid populations at this time of the year (Wiktelius & Ekbom 1985; Chiverton 1986; Wiktelius 1986; Sunderland *et al*. 1987). By contrast, Collembola numbers fell rapidly between 27 May and 9 June. Unlike all previous studies we were able to relate these changes in aphid and Collembola numbers

available at web-sites directly with aphid consumption. During June, following the collapse in Collembola numbers but before aphid numbers had increased significantly, the concentrations of aphid in the guts of the spiders increased disproportionately, especially on 23 June. The evidence suggests that, once alternative prey abundance declined, aphid predation increased, supporting the hypothesis that alternative prey availability directly affects predation rates on target species. Nevertheless, as found by Settle *et al*. (1996), early season dependence on high densities of detritivores would have helped to maintain and retain spiders in the field, which were then able to turn to feeding on the aphid pests once Collembola numbers declined as the season progressed.

Multiple regression models were used to analyse the effects of the presence of alternative prey on the consumption of aphids by spiders. Only Collembola had a significant effect mainly because they were so numerous. The results for Erigoninae showed that Collembola had a significant negative effect on aphid consumption. However, such results must be interpreted with care. Figure 4c,d shows that when there were many Collembola in the field there were few aphids, then later when there were many aphids there were few Collembola. Thus, this negative effect of Collembola could simply be a temporal artefact and nothing to do with prey choice. However, the analysis of the data for Linyphiinae over the same period in the same crop showed no evidence at all of an effect of Collembola on the rate of aphid predation. This suggests strongly that Collembola densities had no effect upon the rates at which Linyphiinae consumed aphids. This is consistent with the trap data in Harwood *et al*. (2003), which found significantly more aphids but fewer Collembola at the web-sites of Linyphiinae. However, as can be seen from Fig. 4a, Collembola were still present in large numbers at the web-sites of Linyphiinae but the latter appeared to have concentrated their feeding efforts on largely non-Collembola food items and instead consumed significant quantities of aphid protein, even when aphid numbers were barely recordable. We made no attempt to measure the relative or absolute densities of spiders in the field, and therefore could not measure their impact on prey populations. However, the Linyphiinae (females) ate greater quantities of aphid (partly because of their size) than the Erigoninae. Aphids were also a greater proportion of the prey in their gut (i.e. the concentration of aphid proteins was higher). Given this, and their relative immunity to the effects of alternative prey (or at least Collembola), the Linyphiinae might be expected, individually, to contribute more to aphid control than the Erigoninae.

Analysis of aphid consumption, and the effects of alternative prey on this, using monoclonal antibodies, revealed ecological processes that could not be detected using population monitoring alone, highlighting the value of such molecular approaches.

Acknowledgements

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